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Measuring the Production of Marine Phytoplankton

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GLOSSARY OF THE MORE COMMONLY USED SYMBOLS AND ABBREVIATIONS

HPPU	Harvey Plant Pigment Unit. (See page 9 for definition.)
SPU	Richards Specified Pigment Unit for plant pigments. (See page 9 for definition.)
MSPU	Milli-SPU (1/1000 of an SPU).
PQ	Photosynthetic quotient. $\frac{+\Delta O_2}{-\Delta CO_2}$. (See page 7 for definition.)
RQ	Respiratory quotient. $\frac{+\Delta CO_2}{-\Delta O_2}$. (See page 7 for definition.)
LB	"Light Bottle". The clear glass bottle used in marine photosynthesis studies; may also symbolize the amount of oxygen evolved photosynthetically in a light bottle.
DB	"Dark Bottle". The blackened opaque bottle used in marine photosynthesis studies; may also symbolize the amount of oxygen consumed by respiration in a dark bottle.
n	The number of unicellular organisms in a unit volume of water.
p	The amount of living cellular substance in a unit volume of water. Generally refers to the amount of organically combined carbon in planktonic algae in the units, mg C/m^3.
k	The growth constant of a unicellular organism in the equations: $\frac{dn}{dt} = k.n$; or $\frac{dp}{dt} = k.p$, when the temperature is specified and conditions of nutrition, etc., are *optimum*.
k'	The growth constant, see k above, applying under any given natural condition, not necessarily optimum.
t_g	The mean division time of a uncellular organism.
P	The rate of photosynthesis (gross or net), as mg C/m^3/hour, under the prevailing conditions of illumination.
P_{max}	The rate of photosynthesis (gross or net) under conditions of *optimum* illumination, which give rise to the maximum possible rate of photosynthesis.
f	The ratio P/P_{max}.
z	The depth of a given location measured in meters, positive from the surface of the sea downwards.
I	The intensity of illumination. In this review I is expressed as the number of gram-calories of radiant energy, in the photosynthetically active spectral range 3800A to 7200A, which falls on 1 cm^2 of surface in 1 minute.

I_z	The intensity of illumination at a depth *z* meters.	
$I_{surface}$	The intensity of illumination at the sea surface.	
$I_{max} = I_{sat}$	The optimum intensity of illumination for photosynthesis, ie. the illumination under which a given algal population photosynthesizes at the maximum rate, P_{max}; known also as the "saturation" light intensity.	
I_{comp}	The "compensation" light intensity at which the average rate of gross photosynthesis in a population just equals the rate of respiration.	
PI	The Productivity Index. This is the rate of photosynthesis per unit phytoplankton crop at a standard light intensity. (See pages 8 and 101 for definitions.)	
D_E	The depth (in meters) of the euphotic zone, ie. the depth of I_{comp}, where gross photosynthesis is just balanced by respiration.	
D_M	The depth (in meters) of the upper stratum of the ocean in which the water has zero or negative stability and is continually mixed by turbulence.	
D_C	The depth (in meters) where the *integral* photosynthesis in a column of water just equals the *integral* respiration in the column.	
R and R_{day}	The integration factors that convert primary production per unit volume per hour under optimum illumination to the total production in the euphotic zone beneath unit area per hour, or per day, respectively. See page 134 for definitions.)	
κ_λ	The vertical extinction coefficient at a wavelength λ used in the Lambert Law: $I_z = I_{surface}\exp.(-\kappa.z)$ where the illumination terms refer to the *vertical* illumination.	
A_d	The virtual coefficient of "diffusion"	(See page 126 for definitions.)
A_h	The virtual coefficient of heat "conduction"	
A_μ	The virtual coefficient of viscosity	
D_d D_h D_μ	The Fick-type transport constants ($cm^2.sec^{-1}$) corresponding to the A terms above. (See page 126 for definitions.)	

INTRODUCTORY REMARKS

The photosynthetic fixation of carbon dioxide to form plant material is probably the most important single factor governing the productivity of any sea region. The quantitative measurement of this fixation is therefore of the greatest importance to fisheries but it is only comparatively recently that this has begun to be fully appreciated. As several persons have remarked, the situation is not unlike that which would prevail if livestock raisers lacked knowledge of, or interest in pasture conditions.

The overall efficiency of the conversion of phytoplankton to pelagic fish is low, being a few tenths of a per cent at most (cf. Kesteven and Laevastu, 1957) but however complicated and significant the ecological relationships in the subsequent food chain may be, the rate and extent at which primary plant food is synthesized must be one of the main factors deciding the relative productivity of various water masses.

Extensive diatom blooms ("weedy water") have an adverse effect on, or are not associated with, a high fish population (ref. e.g. Stevens, 1949) and the occurrence of the wrong kind of plankton at the wrong time can be disastrous for some of the zooplankters (ref. e.g. Knight-Jones, 1950) but such conditions are probably exceptional. In general, there is ample evidence for a direct relation between phytoplankton and fish production.

The correlation of fish abundance with those factors of physical or chemical oceanography that affect the primary fertility of sea water has been discussed by Allen (1909); Russell (1935); Iselin (1939); Cooper (1948); Sverdrup (1955); Uda and Ishimo (1958) to name but a few. The investigations described by Marshall, Nicholls and Orr (1934), Harvey, Cooper *et al.* (1935), Nielsen (1937a) and Marshall and Orr (1952) (ref. also Nielsen, 1958) are sufficient to illustrate the direct relationship between phytoplankton production and the abundance of zooplankton. The phytoplankton serves as direct food for fish larvae and some juvenile fish, such as the sardine, and is said to be the principal food of the pilchard, but the relationships usually described between primary productivity and fish production (cf. Savage and Hardy, 1935; Nielsen, 1935; Hardy *et al.*, 1936; Savage and Wimpenny, 1936; Tham, 1953; Cushing, 1955; Steel, 1957b; Nelson, 1958) are generally via the "secondary productivity" of zooplankton. However, good fishing areas may be an appreciable distance from regions of high primary productivity if strong tides and convergences concentrate the plankton.

The need to measure the standing crop and rate of production of plant material near the ocean surface is now generally recognized to be urgent but there is no general agreement as to the best techniques employed. Laborious and time-consuming methods involving the direct counting and identification of plant cells are clearly impractical (short of a major technicological "breakthrough" in this field) when a large sea area is to be covered in synoptic surveys spaced closely together in time. Methods based on chemical or physico-chemical principles are now employed in these circumstances and it is possible, by such methods, to obtain valuable information about a plant population and its autosynthesis and decay without making a single observation of the individual organisms responsible for photosynthetic activity.

The extent to which taxonomic studies should accompany such work is open to debate. It would be clearly unwise to abandon all descriptive studies and, eventually, the intensive study of individual species will again assume prime importance. The immediate need, however, is for the application of rapid physico-chemical procedures, and the object of this review is to attempt the collection and critical discussion of the widely scattered literature on this subject, a literature characterized, in part, by contradictions, both in experimental data and in their interpretation.

A review of some aspects of primary productivity has been given by Ryther (1956a) and more recently by Nielsen (1957a) and Laevastu (1957). The excellent review by Lund and Talling (1957) covers much of the ground in the field of limnology. However, at the time of writing, the author has seen no comprehensive and critical review of marine primary productivity that serves as an adequate introduction to the subject in all its aspects for workers about to commence active research in this field. Such a review seems to be worthwhile in view of the rapid changes in techniques, methods, and ideas which we are now witnessing. The literature coverage in the present work is far from complete and, because of relative inaccessibility and translation difficulties, only a few of the contributions of Russian marine science are discussed. It is not the purpose of the review, however, to compile a record of all the field work ever undertaken on this subject; references to such work have only been included when they illustrate the application of a method or lead to data of general interest. It is hoped that, in this context, few serious omissions will be found and the review will provide a useful entry to the literature.

The part played by light in primary productivity has already been reviewed in detail (Strickland, 1958) and only those aspects having direct bearing on productivity *measurement* will be discussed again. Similarly, the role of plant physiology and nutrition is only mentioned in the present work when of direct relevance to productivity measurements. No detailed account of the complex phytoplankton-zooplankton interrelationships has been attempted. Although no worker in the field of primary productivity measurement can ignore for long the predation factors affecting plant communities, the measurement of standing crop and the measurement of the rate of plant autosynthesis can both be undertaken without reference to the associated zooplankton population.

The terminology used by many planktologists is ponderous and precise definitions are sometimes lacking. A self-consistent terminology and set of definitions, applicable to marine primary productivity, will be given in the first section of this review. Although in no way authoritative, these definitions are thought to be as satisfactory as is feasible in the context of the work under discussion. All data from the literature will be expressed in the units defined in Section I, in the hope that this will make for greater clarity. A list of the interconversion factors used is given for the assistance of the reader.

The classification used for the algae is taken from the Manual of Phycology (Smith, 1951). Throughout this review it must be borne in mind that nearly every reference to the diatom *Nitzschia closterium* should, in fact, be applied to the species *Phaeodactylum tricornutum,* with which there has been confusion for many years. A list of the papers in which this confusion occurs is given by Lewin (1958). As *P. tricornutum* is in no manner a typical marine diatom and, in fact, can be considered to be in a separate sub-order of the Bacillariophyceae, great care is necessary when generalizing from the behavior and properties of this alga to marine diatoms in general.

The opportunity is taken in this review to discuss the chemical composition of planktonic algae in some detail as this is relevant to many of the methods suggested for standing crop measurement. However the material in this section of the work is in no sense comprehensive. Similarly, a short account is given of the kinetics of unicellular growth, as background material for those not very familiar with the subject, but the treatment is only introductory.

It is almost impossible to write any detailed account of the composition, metabolism and properties of marine planktonic algae without making some reference to work reported on freshwater species and to experiments carried out by limnologists. In some instances such work comprises the only available information that may have a bearing on certain marine problems. Generalizations from freshwater to marine algology, however, must always be viewed with reserve. If the present author is guilty of such generalizations he is, at least, not alone in this practice.

SECTION I. DEFINITIONS AND CONVERSION FACTORS

A. DEFINITIONS

1. PLANKTON TERMINOLOGY

a. THE PLANKTON (individually, PLANKTERS)

Organisms suspended in water, without mobility or with very limited mobility, which cannot or do not maintain their distribution against the effects of local water movements. The terms *phytoplankton* and *zooplankton* are used for the plant and animal subdivisions of these organisms respectively. In borderline cases an organism should be classed as a phytoplankter (for productivity considerations) if it is capable of any photosynthetic activity.

b. THE NEKTON

Organisms capable of sufficient mobility to maintain their distribution against local water movements. The definitions of plankton and nekton are not sufficiently precise for the unambiguous classification of certain organisms under certain conditions but, in general, the terminology is adequate.

c. DETRITUS

Suspended matter of organic origin permanently incapable of reproduction. The prefix phytoplankton, zooplankton or nekton can be used to indicate the exact origin of the detritus but, if unqualified, the term normally implies phytoplankton detritus with perhaps some zooplankton detritus, mainly as faeces. Phytoplankton detritus can be conceived of as being capable of some limited autosynthesis but not reproduction to form new organisms and, in this sense, is "dead". Suspended mineral matter is best described as such and not termed detritus, although organic detritus may often "collect" a considerable amount of inorganic material.

d. TERMINOLOGY USED TO DESCRIBE THE LOCATION OF PLANKTON

The term *euphotic zone* is used in this review to describe the surface layer of the ocean in which, under the conditions stated, the net rate of photosynthesis of phytoplankton is positive. The depth of this zone is governed mainly by the mean amount of solar radiation and the optical properties of the water.

The term *oceanic* is used for plankton located outside coastal waters and *neritic* for the plankton of coastal areas. It is not clear whether the generally accepted definition of the oceanic province, as that area where the water depth is greater than about 200 m, applies too well when discussing phytoplankton. Metabolites, characteristic of inshore waters, may be transported well into the "oceanic" area if there is little or no continental shelf and if offshore currents are strong. From the standpoint of primary productivity a truly oceanic area is probably at least 200 to 300 miles from the nearest part of the continental shelf.

e. TERMINOLOGY USED TO DESCRIBE THE ABUNDANCE OF PLANKTON

Several rather ill-defined terms, used mainly in limnology, will classify waters according to their content of living matter and biotic potential. Only the terms *eutrophic* and *oligotrophic* are used in this review to describe marine conditions, and these indicate the extremes of plankton abundance from very fertile areas (generally coastal waters with pronounced upwelling) to sparsely populated waters (characterized by the tropical oceans).

It is sometimes useful to classify according to the numbers of species present in the plankton. The terms *monomictic, polymictic* and *pantomictic* have been used to indicate that the plankton consists either essentially of one species (90% or more), or of several species in approximately the same abundance (with other species much less prevalent), or that no one species is particularly prevalent in a population containing very many species.

f. TERMINOLOGY USED TO DESCRIBE THE ORIGIN OF PLANKTON

The terms *endemic* and *exotic* applied to plankton or detritus indicate that it was produced in the region under study or elsewhere, respectively. These terms are not readily defined with precision.

g. TERMINOLOGY USED IN THE SIZE CLASSIFICATION OF PHYTOPLANKTON

For phytoplankters the terms *microplankton, nannoplankton* and, rarely, *ultraplankton* are all that need be used, indicating a size distribution of organisms approximately in the ranges 50 to 500 microns, 10 to 50 microns and 0.5 to 10 microns, respectively, for the largest dimension.

h. TERMINOLOGY USED TO DESCRIBE THE NUTRITIONAL REQUIREMENTS OF PHYTOPLANKTERS

The term *autotrophic* is reserved for those phytoplankters that require no organic material whatever for their normal growth and reproduction. *Auxotrophic* organisms can obtain most of their growth requirements from inorganic sources but have a definite requirement for certain organic molecules and without these cannot grow or reproduce in a normal manner. *Heterotrophic* plants may obtain their constituent carbon, and perhaps also the nitrogen, sulphur and phosphorus they need, exclusively from organic sources. Many algae may be heterotrophs in the dark but behave mainly as autotrophs in the light.

2. PRODUCTIVITY TERMINOLOGY

a. PHOTOSYNTHETIC RATE

The rate of photosynthesis can be defined in at least three ways, according to the method of measurement used.

(i) The rate of increase of dry organic matter in plants *that results from photosynthetic activity.* This definition is rarely employed in work with algae as it is not often practicable to measure such a weight increase or to decide what fraction of the weight increase arises from photosynthesis and how much from heterotrophic growth.

(ii) The rate of increase of the amount of oxygen in the extra-cellular environment of plants *that results from photosynthetic activity.* This rate of increase is not necessarily the same rate as measured in (i), above, even on a molecular basis, as the plant material may not liberate all photosynthetically formed oxygen immediately and some uptake of carbon dioxide may occur without simultaneous oxygen liberation. However, for periods exceeding a few minutes, a pseudo-equilibrium stage will probably be reached and the two measurements will be proportional to each other and related, in principle, by a suitable empirical factor.

(iii) The rate of decrease of the amount of carbon dioxide from the extra-cellular environment of plants *that results from photosynthetic activity.* This rate of decrease is not necessarily the same rate as in (i) or (ii), above, as the carbon dioxide taken in by a photosynthesizing system may not all be used at once or may only produce organic compounds of transient stability. However, for periods exceeding a few minutes, provided that a 'dark uptake' blank is deducted, the measurements will be proportional and related by some factor.

The terms *gross* and *net* are used in conjunction with photosynthesis to distinguish between the true synthesis of organic matter resulting from exposure to light (gross) and the net formation of organic matter that is found after allowance has been made for the respiration and other losses that occur simultaneously with the photosynthetic processes.

b. PHOTOSYNTHETIC QUOTIENT (PQ) AND RESPIRATORY QUOTIENT (RQ)

These quotients are dimensionless numbers indicating the relative amounts of oxygen and carbon involved in the processes of photosynthesis and respiration.

$$\text{Photosynethic quotient} = PQ = \frac{\text{Molecules of oxygen liberated during photosynthesis}}{\text{Molecules of carbon dioxide assimilated}} = \frac{+\Delta O_2}{-\Delta CO_2}$$

$$\text{Respiratory quotient} = RQ = \frac{\text{Molecules of carbon dioxide liberated during respiration}}{\text{Molecules of oxygen consumed}} = \frac{+\Delta CO_2}{-\Delta O_2}$$

c. PRIMARY PRODUCT OR PRIMARY STANDING CROP

(dimensions: $M.L^{-3}$ or $M.L^{-2}$)

The instantaneous value of the amount of living plant material present in water. It will be here measured on the basis of the organic carbon (in contrast with any carbonate carbon) present in unit volume or beneath unit area of sea surface. The units that will be used in this review are restricted to:

$$\text{mg C/m}^3 \qquad \text{and} \qquad \text{mg C/m}^2$$

d. Net primary productivity[1] (dimensions: $M.L^{-3}.T^{-1}$ or $M.L^{-2}.T^{-1}$)

The net rate of autosynthesis of the organic constituents of plant material in water. This will again be measured on the basis of organic carbon per unit volume or beneath unit area of sea surface, with the time scale chosen to suit a particular context. The definition is limited to the primary productivity of plant material and, by virtue of the methods used, generally refers to productivity from inorganic sources of carbon. The measurement does *not* include the rate of loss of primary material by death or predation. The units that will be used in this review are restricted to:

$(mg\ C/m^3)/hour$; $(mg\ C/m^3)/day$; $(mg\ C/m^2)/day$; $(g\ C/m^2)/year$.

The definition of day is not always clear in the literature but may be of considerable importance, especially when considering oligotrophic waters. When used alone in this review the word "day" generally refers to the hours of daylight during which a net photosynthesis occurs in the sea surface water. When a full 24-hour period is implied the term $day_{(24)}$ is used.

e. Gross primary productivity

The definition and units are the same as those used above for net productivity except that the rate under consideration is the *gross* rate of the photosynthetic increase in plant crop before any corrections for respiration or excretion are made. The gross productivity is of restricted interest when studying long-term food production trends.

f. Net production rate

This term should be reserved to describe the rate of production (either positive or negative) of plant organisms under the influence of *all environmental factors* and thus include losses by death and predation. The units used may, for convenience, be made the same as those used to describe net primary productivity but the two measurements are quite different and should not be confused.

g. Productivity index (dimensions: $T^2.M^{-1}$)

The net primary productivity per unit primary standing crop at some standard light intensity is a direct index of the fertility of a given water mass and its endemic crop of phytoplankters. The index will be defined here with 1 langley/min (3800-7200 A) as standard illumination. Severe photoinhibition would occur, in practice, at an illumination of this magnitude, but the index refers to a hypothetical rate that would result were photosynthesis to continue to increase with light intensity at the same rate as that found below the optimal illumination of some 0.1 ly/min. The unit that will be used in this review is:

$$(hour)^{-1}.(ly/min)^{-1}$$

The productivity index under natural light conditions has been termed the "turnover rate" (ref. Cushing *et al.*, 1958)

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[1] The term "primary production" is, perhaps, preferable but both "production" and "productivity" are in common usage to describe this concept.

3. PLANT PIGMENT TERMINOLOGY

It is unfortunate that complications should be introduced in the definition of units used for measuring the amount of plant pigment but, historically, much work was undertaken before purified plant pigments were readily available as standards. Wherever possible, the weight of pure pigment is now quoted, the unit generally being the *milligram*.

a. HARVEY PLANT PIGMENT UNIT (HPPU)

This unit is no longer employed, but for over a decade many standing crop measurements of phytoplankton were expressed in the HPPU, which has therefore assumed considerable importance.

One HPPU of phytoplankton, when dissolved in 80% aqueous acetone, matches, visually, the intensity of colour found in a weakly acid solution of 430 micrograms of nickel sulphate hexahydrate and 25 micrograms of potassium chromate in water of the same volume (Harvey, 1934a). The definition given by Harvey (1950), in a footnote, is at variance with the above definition and contains, presumably, a misprint.

b. RICHARDS SPECIFIED PIGMENT UNITS (SPU)

These units (Richards with Thompson, 1952) correspond to *about* one gram of the pigment concerned and the milliunit (MSPU) is normally used, approximating to a milligram of pigment. As pure pigment samples of known weight were not used, the weight of one SPU varies. The SPU is defined on the basis of the extinction coefficient at the wavelength of maximum absorbency in 1 liter of 90% aqueous acetone, according to the following table:

Compound	λ_{max} (Angstrom)	E_{1cm}
Chlorophyll *c*	4450	83.5
Neofucoxanthin A	4475	251
Neofucoxanthin B	4460-4480	251
Fucoxanthin	4485	251
Diatoxanthin	4510	251
Diadinoxanthin	4445	251
Astacin-type (Crustacea) carotenoids	4750	251

B. CONVERSION FACTORS

The conversion factors needed for productivity studies may be conveniently divided into two main groups, those required for photosynthetic rate measurements and those commonly employed when considering the standing crop of phytoplankton.

Within each group three distinct types of factor exist. The first type involves the interconversion of dimensions and molecular properties and these factors are, from the standpoint of productivity studies, so precise that they may be regarded as "exact". The second group of factors relates experimentally determined quantities or is obtained from both experimental and "exact" data. These factors incorporate experimental errors and, what is more important, they show only the mean value of properties that have an inherently large standard deviation due to natural variations found with an algal population. When such properties as cell volume are involved the potential variations are so enormous that "average" factors have very little meaning. Finally, factors are often quoted which turn out to be derived from the combination of two or more factors of the second kind mentioned above and then potential errors can become alarming. These derived data may still be of value in certain instances, provided that the uncertainties are fully realized, but often factors of this type have been used without appreciating, or at least without stating, the probable errors involved.

The classic table of "plankton equivalents" was given by Fleming (1939b); see also Sverdrup *et al.* (1942, p. 929). A revised version has been computed by Laevastu (1957). Although these authors indicate some of the uncertainties involved, time tends to lend authority to the printed figure and the uncritical use of factors presented in this form offsets much of their value. It is to be regretted that the International Council (ref. Cushing *et al.*, 1958) should have added their authority to such practice.

In the present review an attempt has been made to classify conversion data according to the uncertainties involved and to give some indication of likely errors. As we are here considering both crop and productivity rates in terms of organically combined carbon, the relationship of other properties to the amount of combined carbon is stressed. The three types of factor are termed "exact", "experimental" and "secondary" to indicate a decreasing order of precision.

1. RATE FACTORS

Although many of the factors used in rate studies can be obtained from a simple differentiation of the standing crop factors given later, the following are primarily used for rate studies alone.

a. EXACT RATE FACTORS

Carbon assimilated by photosynthesis in mg per unit time:

= (mg-at CO_2 carbon assimilated in unit time) × 12

= (mg CO_2 assimilated in unit time) × 0.273

= (ml (N.T.P.) CO_2 assimilated in unit time) × 0.536

= (mg-at O evolved in unit time) $\times \frac{12}{PQ}$

$$= (\text{mg O evolved in unit time}) \times \frac{0.375}{PQ}$$

$$= (\text{ml (N.T.P.) } O_2 \text{ evolved in unit time}) \times \frac{0.536}{PQ}$$

Carbon lost by respiration in mg per unit time:

$= (\text{mg-at } CO_2 \text{ carbon respired in unit time}) \times 12$

$= (\text{mg } CO_2 \text{ respired in unit time}) \times 0.273$

$= (\text{ml (N.T.P.) } CO_2 \text{ respired in unit time}) \times 0.536$

$= (\text{mg-at oxygen consumed in unit time}) \times 12 \times (RQ)$

$= (\text{mg oxygen consumed in unit time}) \times 0.375 \times (RQ)$

$= (\text{ml (N.T.P.) } O_2 \text{ consumed in unit time}) \times 0.536 \times (RQ)$

No one value for PQ or RQ can be assumed to be universally applicable although these ratios are often taken to be unity. Values of 1.2 and 1.0 for PQ and RQ respectively are better and should be used unless the precise values are known (see Section IV.A).

b. EXPERIMENTAL RATE FACTOR

There is now evidence (see Section IV.G) that *Gross* Primary Productivity is a crude function of the chlorophyll *a* content of the living phytoplankton in a water sample. The relationship may be expressed as:

$$\text{Gross primary productivity (mg C/m}^3\text{)/hour} = (30 \pm 20) \times I \times (\text{mg chlorophyll } a/\text{m}^3)$$

where I is the intensity of daylight illumination in ly/min (3800-7200A).

This expression should only be used to estimate gross primary productivity per unit volume and then only when I is below the "optimum" of about 0.1-0.15 ly/min.

Values for the factor can be anywhere between 10 and 100 with natural populations and there is some doubt as to whether the figure 30 has the significance of a "mean" value when studying any particular sea area. However, the use of a factor of 30 should enable an estimate to be made of the gross primary productivity of sea water to within say ±70%, which may be of some value, especially in oligotrophic areas, when only plant pigment data are available. The above relationship tells nothing about the *Net* Primary Productivity of an area, unless respiration is known to be of minor importance.

c. SECONDARY FACTOR

As an appreciable amount of the literature between 1935 and 1950 contains pigment data in HPPU and not as chlorophyll, the gross productivity relationship given above cannot be used. The following equation for use with HPPU is even less precise but may have some value when examining some of the earlier data to determine a likely *order of magnitude* for gross photosynthesis.

$$\text{Gross primary productivity (mg C/m}^3\text{)/hour} = (9 \pm 6).10^{-3} \times I \times (\text{No. of HPPU/m}^3).$$

2. STANDING CROP FACTORS

These have deliberately *not* been collected into one large table in an endeavour to retain better presentation of the relative accuracy of each factor.

a. EXACT STANDING CROP FACTORS

$1\ \mathrm{m}^3 = 1000$ liters

1 mg or mg-atom/$\mathrm{m}^3 = 1 \mu g$ or μg-atom/l

1 micron $(\mu) = 10^{-3}$ mm $= 10^{-4}$ cm; 1 cm $= 10^4$ microns (μ)

1 micron^2 $(\mu^2) = 10^{-6}$ mm^2 $= 10^{-8}$ cm^2; 1 cm^2 $= 10^8$ microns^2 (μ^2)

1 micron^3 $(\mu^3) = 10^{-9}$ mm^3 $= 10^{-12}$ ml: 1 ml $= 10^{12}$ microns^3 (μ^3)

Assuming phytoplankton cells have a specific gravity of unity,

$10^9 \mu^3$ of phytoplankton weigh 1 mg.

b. EXPERIMENTAL AND SECONDARY STANDING CROP FACTORS

In what follows, experimental standing crop factors are denoted by (E), and secondary standing crop factors are denoted by (S). Factors have been collected in the groupings thought to be most useful in practical calculations.

(i) *Calculation of standing crop from nitrogen or phosphorus data*

The total carbon in phytoplankton can be estimated, if either the phosphorus or nitrogen content is known, from the ratio C:N:P in these plants. No other elements are thought to be suitable for this purpose and, as the phosphorus content of algae can vary up to 5-fold according to the state of nutrition of the cells, it will be realized that standing crop estimates from N or P data can be only *very approximate*. The ratio of nitrogen to phosphorus is less variable (about 5.5:1 to 7.5:1, with a mean of 6.5:1) and has been the subject of much investigation; but surprisingly little work has been undertaken to measure the ratios C:N or C:P directly and they must often be inferred from other analyses. Fuller details will be found in Section II.

The variation of the ratio of organically combined carbon to Kjeldahl nitrogen or organically combined carbon to organically combined phosphorus is so large that no distinct trend of the ratios with species is obvious and values are probably more a function of the physiological state of the plankton at the time of measurement. The published data suggest that only one factor is justified for the marine phytoplankton, in the absence of special knowledge of the phytoplankters concerned, namely:

$$\text{mg C} = \text{mg N} \times (6 \pm 2) \quad \text{(S)}$$
$$\text{mg C} = \text{mg P} \times (40 \pm 15) \quad \text{(S)}$$

For pure Chlorophyceae the phosphorus factor is definitely lower and may be nearer to 25 (Ketchum and Redfield, 1949).

(ii) *Calculation of standing crop from pigment data*

As the HPPU is a rather imprecise measure of the chlorophyll content of phytoplankton it may be as well to relate this unit to "chlorophyll" before going further. The experimental conversion factors quoted in the literature vary rather widely, due mainly to the subjective nature of the HPPU measurement. Nearly 10-fold variations have been quoted but probably the following relationships are applicable to most published data:

$$\text{No. of HPPU's} = (3300 \pm 1500) \times \text{mg chlorophyll} \quad \text{(E)}$$
$$\text{mg chlorophyll} = (3 \pm 1 \times 10^{-4}) \times (\text{No. of HPPU's}) \quad \text{(E)}$$

(see Riley, 1941b; Atkins and Parke, 1951; Riley *et al.*, 1956; Krey, 1957). The value quoted by Harvey (1950) of 1 HPPU being equivalent to 3 μg chlorophyll, which has been used by Gillbrich (1952), is, presumably, a misprint.

There are very wide differences in the literature for the stated values of the chlorophyll content of phytoplankton. As the amount of this pigment can vary in most algae by a factor of at least 5, according to the prehistory of nutrition and illumination, it is not surprising that such disagreements occur, and matters are complicated by the inclusion of non-pigmented detritus in some measurements. Nowhere does there appear to have been any systematic measurement of pigment and total carbon by combustion, to determine this ratio directly on cultures of pure phytoplankters or on mixed natural populations. Banse (1956) has discussed some literature values and their derivations.

All factors are "secondary" and, except for relationships that can be deduced from the correlation of HPPU or chlorophyll to nitrogen and phosphorus, the factors originate from measurements of the percentage of chlorophyll in wet or dry algae, together with assumptions as to the amount of carbon per unit algal weight or volume (see later). Robinowitch (1945) quotes values for the chlorophyll content of terrestrial plants between about 0.2% and 0.8% and similar results are given for brown algae. Green algae *(Chlorella)* may contain some 2% and as high as 4% to 5% under exceptional conditions. If

$$\text{mg C} = \text{F} \times \text{mg chlorophyll} \quad \text{(S)}$$

where C is the organically combined carbon and "chlorophyll" is understood to be either chlorophyll *a* or a mixture of chlorophyll *a* and *b*, we find that values for F derived from literature data vary over at least an order of magnitude. The diatoms are stated to have nearly twice the chlorophyll content of the dinoflagellates on a *cell volume* basis (Gillbricht, 1952). However, one of the main uncertainties in most calculations is the weight of carbon per unit volume of phytoplankton and, as a result, no differences in the factor F can be related with certainty to differences of class.

The following values for F have been calculated, each value having a considerable margin of uncertainty:

Nature of plankton	Mean F value	Key measurement	Reference to key data
Dunaliella euchlora	45	From mean chlorophyll:N ratio	Yentsch, private comm.
Mixed lake population	50	From HPPU and dry weight	Riley, 1938b
Mixed population	61	From HPPU and cell volume	Riley, 1941b
Mixed population	20	From HPPU and phosphorus	Harvey, 1950
Chlorella sp.	6	Pigment per cell volume	Atkins and Parke, 1951
Coscinodiscus centralis	4	"	"
Thalassiosira gravida	70	"	"
Diatoms	17	"	Gillbricht, 1952
Gymnodinium sp.	12	"	Atkins and Parke, 1951
Dinoflagellates	33	"	Gillbricht, 1952
Chaetoceros gracilis	1.1?	"	Krey, 1939
Chlorella sp.	16	Pigment per unit dry weight	Data from Rabinowitch, 1945
Nitzschia closterium	13	"	Pace, 1941
Coscinodiscus sp.	70	"	Riley *et al.*, 1956
Mixed population	66	"	Riley, 1941b
Gonyaulax sp.	115	"	Riley *et al.*, 1956
Mixed population	60 (30-130)	"	Riley *et al.*, 1956
Mean value for marine phytoplankters	35 (20-70)	"	Seybold and Egle, 1938
Mixed lake population	60	"	Wright, 1959
Mainly coccolithophores from culture of ocean crop	48	Ratio of carbon increase to pigment increase	Unpublished data in author's laboratories

It is obvious that no value for F can be quoted that has any significance to other than an order of magnitude. This applies even if cell volume or dry weights are to be calculated. The value for F doubtless depends mainly on the species, location and state of nutrition of the plankters.

As a working rule, in the absence of much needed experimental data, it is suggested that a value of $F = 30$ be used for cultures or natural populations known to be without nutrient deficiencies and $F = 60$ for mixed natural populations subject to high light intensities or in warm nutrient-deficient waters. In neither case can the resulting estimate of standing crop be assumed to be correct to better than a factor of about *0.3 to 3*.

In some circumstances the ratio of combined phosphorus or nitrogen to chlorophyll may be less variable than the ratio of carbon to chlorophyll and the equations:

$$\text{mg P} = (0.75 \pm 0.2) \times \text{mg chlorophyll (E)}$$

and

$$\text{mg N} = (7 \pm 3) \times \text{mg chlorophyll (E)}$$

are suggested as applicable to most populations with the uncertainties indicated.

In the Chlorophyceae the carotenoids amount to some 0.1-0.4% of the dry algae (Seybold and Egle, 1958, Haskin, 1942). The carotenoid pigments in *Nitzschia closterium* are given by Pace (1941) as 0.64%, indicating that:

$$\text{mg C} = (\text{40 approx.}) \times \text{mg plant carotenoid pigments (S)}$$

It is unfortunate that more attention has not been paid to the carotenoids in phytoplankton, as the weight of these pigments almost certainly bears a more constant ratio to the weight of organic matter than is the case with the chlorophylls.

Several estimates have been made of the number of cells that contain unit weight of chlorophyll. No average figure has any significance but the following estimates for individual genera or species may be useful. In the expression:

$$\text{No. of cells} = F \times 10^{6} \times \text{mg chlorophyll}$$

the following values for F have been given:

Plankter	F value	Reference
Chlamydomonas	150	Atkins and Parke, 1951
Chlorella sp.	2500	Atkins and Parke, 1951
Coscinodiscus radiatus	13	Riley *et al.*, 1956
Coscinodiscus centralis	0.075	Atkins and Parke, 1951
Chaetoceros gracilis	0.3	Krey, 1939
Chaetoceros vanheurckii	170	Graham, 1943
Thalassiosira gravida	125	Atkins and Parke, 1951
Nitzschia seriata	1250	Graham, 1943
Phaeodactylum tricornutum (?)	750	Atkins and Parke, 1951
Nitzschia closterium	1800	Pace, 1941
Gymnodinium sp.	58	Atkins and Parke, 1951
Gonyaulax sp.	45	Riley *et al.*, 1956

(iii) Volume and weight relationships in the phytoplankton

DRIED PHYTOPLANKTON WEIGHT TO WET WEIGHT AND VOLUME. Two "wet weights" must be recognized, the true wet weight of the cells themselves with no extraneous water and the experimental wet weight obtained after draining the cells in some standard manner. The first weight is obtained from algal cell volumes, as measured microscopically, and a specific gravity value which, for all practical purposes, may be taken as unity. To avoid confusion this quantity should be called, simply, *algal weight*. The experimental *"wet weight"* will vary considerably according to the technique employed and will rarely, if ever, be less than twice the true algal weight, due to the presence of interstitial water. The confusion of these two wet weight figures by some authors has caused serious errors when computing, for example, chlorophyll:carbon ratios from cell volumes.

In the expression:

$$\text{mg dry weight} = F \times \text{mg wet weight (E)}$$

where the dry weight is obtained by oven drying at about 50-100° C to constant weight, F values have been used which vary from about 0.1 to 0.25 (Brandt and Raben, 1920; Grim, 1939; Myers, 1946a; Atkins and Jenkins, 1953; Burlew, 1953; Riley *et al.*, 1956) and no one value has any more merit than another as they depend upon the techniques used. The application of a mean value of, say, $F = 0.2$ cannot be more precise than ± 50%.

For the expression:

$$\text{mg dry weight} = F \times \text{mg algal weight (E)}$$

where:

$$\text{mg algal weight} = \text{algal cell volume (mm}^3) \times (1.00 \pm 0.03)$$

the values for F should show considerably less variation and be less subjective. Unfortunately, however, no convincing data have been reported. From the work of Grim (1939), Riley (1941b), McQuate (1956), Cushing (1957b) and Ketchum and Redfield (1949) one would hazard a value of $F = 0.35$ but the precision is very poor. This situation could easily be remedied by further experimentation.

ASH-FREE PHYTOPLANKTON WEIGHT TO DRY PHYTOPLANKTON WEIGHT. The ash of many marine algae does not exceed some 10% of the dry weight and consists mainly of the common seawater electrolytes. For the Xanthophyceae and Chrysophyceae the percentage may be a little higher, owing to the presence of calcareous and siliceous material and, with diatoms, a considerable amount of silica is to be expected. In most analyses results are probably high owing to the unavoidable inclusion of extraneous inorganic matter in the ash determinations.

In the expression:

$$\text{mg ash-free organic matter} = F \times \text{mg dry weight (E)}$$

F would be expected to differ according to the class of algae and, with the Bacillariophyceae, according to the degree of silicification of the plants, which can vary considerably. Consideration of the values given by Brandt and Raben (1920), Riley (1941b), Vinogradov (1953), Ketchum and Redfield (1949), Low (1955) and Riley *et al.*, (1956) leads to the following suggestions:

For green algae	$F = 0.85 \pm 0.05$
For dinoflagellates	$F = 0.8 \pm 0.1$
For diatoms	$F = 0.5 \pm 0.1$

with $F = 0.6 \pm 0.2$ for most mixed populations in subarctic regions if they are substantially free from extraneous inorganic particles.

Again, with this factor, further direct experimentation could give useful results and is long overdue.

ORGANICALLY COMBINED CARBON TO ASH-FREE ORGANIC MATTER. The theoretical value for F in the expression:

$$\text{mg C} = F \times \text{mg ash-free organic matter} \quad (E)$$

is 0.4 for a pure hexose, about 0.52 for proteins and about 0.75 for 'fats'. The values reported for phytoplankton (cf. the collection of data by Ryther, 1956a) and indeed most marine creatures, are surprisingly constant around 0.5 and F may be taken as 0.5 ± 0.05 with little error.

ORGANICALLY COMBINED CARBON TO ALGAL VOLUME OR WEIGHT. A consideration of the above factors suggests that F in the expression:

$$\text{mg C} = F \times \text{mg algal weight (or mm}^3\text{ algal volume)} \quad (S)$$

should lie, very approximately, in the range 0.09-0.15 (see also Cushing, 1957a). It is gratifying to note that the direct experimental value reported by Cushing and Nicholson (1957) for *Skeletonema costatum* is 0.13. This value was used in calculating some of the chlorophyll/carbon data given above.

BIOMASS TO CELL VOLUME. There seems to be little point in trying to report such data for each species (cf. Laevastu, 1957). In practice these factors will only be used after having counted the algal cells in a sample of sea water, in which case an exact estimate of the cell volumes concerned might just as well be made at the same time for use in the subsequent calculations.

SECTION II. CHEMICAL COMPOSITION OF PHYTOPLANKTON

A. ELEMENTARY COMPOSITION

A knowledge of the elementary composition of phytoplankton is of interest, from the point of view of productivity measurements, in as far as it enables one to identify and estimate the amount of primary product. For all practical purposes the primary product, or standing crop, in arctic and subarctic areas may be considered to consist of species from the classes Bacillariophyceae and Dinophyceae, with some genera of the Chlorophyceae, Chrysophyceae, Myxophyceae and Xanthophyceae being of occasional importance.

For reasons of analytical technique, the phytoplankton crop can only be determined by means of those elements which are highly concentrated by the marine plants from out of the surrounding sea water. Thus sodium and chlorine, although present in the plants in quantities of up to 10% or more on a dry weight basis, are not suitable elements and the same argument applies to a lesser extent to calcium and sulphur. Iron is concentrated by most marine algae, where it may accumulate to the extent of several per cent, but unfortunately iron is also present in the surrounding sea water in an inorganic particulate form and thus collects and contaminates the phytoplankton on filtration.

We are left, therefore, with the elements silicon, nitrogen, phosphorus and carbon with which to attempt the measurement of the standing crop of plant material in sea water.

1. SILICON

The silicon content might well be of some use in assessing the contribution of the diatoms to the total phytoplankton crop (cf. Aleem, 1949). With the exception of the ubiquitous, but rarely abundant, Silicoflagellates and some weakly silicified Xanthophyceae and Chrysophyceae, it is unlikely that the Si:C ratio in marine algae, other than the diatoms, exceeds 0.01 to 0.05. The ratio in the diatoms, on the other hand, is much higher, because of the wall of amorphous hydrated silica that is found outside the "pectin" layer comprising the cell walls of these plants (Liebisch, 1929; Rogall, 1939). Unfortunately, however, the silicon content of diatoms is nowhere near constant, being generally greater in the Pennales and in benthic forms than in the Centrales and in oceanic forms. The degree of silicification depends on the amount of silicon available to the diatoms from the surrounding water and other factors and can vary at least 5-fold (Lewin, 1957; Jorgensen, 1955). In natural marine populations the Si:C ratio varies from about 0.4 to nearly 1 (calculated from data given by Vinogradov 1953) but is probably near to 0.8 in sea areas where silicon is not a 'limiting' nutrient. The main drawback to the use of silicon as an indicator of the diatom population, however, is practical. Inorganic sources of the element, in the form of clay, sand, etc., will contaminate most coastal standing crop samples to a serious extent. It should be possible, however, to devise some form of chemical attack, say strong aqueous alkali, which would bring about the dissolution of diatom frustules without having too great a reaction with sand and other inorganic sources of silicon. Such a technique merits investigation.

2. PHOSPHORUS

The rate at which phosphorus is absorbed by growing phytoplankton and the phosphorus content of the resulting cells depends upon the phosphorus and nitrogen content of the surrounding medium. Some of the phosphorus is quite loosely bound and labile in the plant cells. Plants also have the ability to store the element in excess of their normal requirements. The storage products thus formed break down and liberate excess phosphorus during vigorous cell division (Ketchum, 1939a; Scott, 1945; Matsue, 1949; Goldberg *et al.*, 1951; Spenser, 1954). Thus the final phosphorus content of an algal cell depends upon the growth history of the plant and the nutrient properties of the growth medium. A deficiency can develop, especially if nitrate is still present in excess, until the phosphorus content of a cell is only some 20% of its normal value, giving at least a 5-fold variation in the amount of element present. Much of this deficiency can be made up in the dark and complete recovery occurs in the light directly phosphate is added to the medium (Ketchum, 1939b; Scott, 1943; Ketchum and Redfield, 1949).

The ratio of nitrogen to phosphorus in phytoplankton has been the subject of much investigation, mainly because of the significance of this ratio in relation to the availability of the two elements in sea water. Early values for the ratio in plankton are collected by Redfield (1934) and the subject was reviewed by Cooper (1937), who reported a mean value of near to 7:1. The ratio was measured in *Coscinodiscus excentricus* by Cooper (1938) and found to be 5.5:1. Other values can be calculated from the data given in Table I. The ratio is generally in the range 5.5:1 to 7.5:1 but the Chlorophyceae studied by Ketchum (1939b) appear to have a rather low ratio of near to 3:1.

Some values for the phosphorus content of phytoplankton have been collected in Table I from which C:P ratios can be calculated. There are very few analytical data of unquestionable quality to be found for the marine phytoplankton and much of the work has been carried out on the Chlorophyceae or on non-planktonic forms of algae. Many values quoted freely in the literature originate from data obtained well over a quarter of a century ago and one is left in considerable doubt as to the purity of the samples used. The analyses given in Table I have little value other than to illustrate magnitudes and show the large variations possible.

As mentioned in Section I, factors based on 'representative' analyses of phytoplankton have a wide range of uncertainty and should be used with caution.

Like the yeasts, algae (certainly *Chlorella*) store a considerable amount of phosphorus as inorganic polyphosphates (see for example Wassink, 1955; Arnon, 1956), but these compounds have not, as yet, been determined separately during elementary analyses.

TABLE I. Some analytical data for carbon, nitrogen and phosphorus in phytoplankton. (Percentages in dry material.)

Phytoplankter	Carbon	Nitrogen	Phosphorus	Reference
	%	%	%	
Bacillariophyceae				
Skeletonema sp.	25.27	3.84	...	Brandt and Raben, 1920.
Mixed	22.04	5.40	...	Brandt and Raben, 1920.
Coscinodiscus sp.	...	2.19	1.27	Vinogradov, 1953.
Coscinodiscus sp.	20 (approx.)	4.53	0.66	Riley *et al.*, 1956.
Nitzschia closterium	44	5.35	0.83	Ketchum and Redfield, 1949.
Dinophyceae				
Ceratium tripos	33.17	7.19	0.37	Brandt and Raben, 1920.
Peridiniae	40.52	3.25	...	Vinogradov, 1953.
Gonyaulax	37 (approx.)	4.14	0.37	Riley *et al.*, 1956.
Chlorophyceae				
Scenedesmus sp.	46.4	7.1	2.7	Ketchum and Redfield, 1949.
Scenedesmus sp.	...	2.2–7.7	1.0–2.0	Krauss, 1956.
Chlorella	51.4	...	...	Krauss, 1956.
Chlorella (normal culture)	48.5	6.7	2.2	Ketchum, 1939b.
Chlorella (average)	48.3	6.6	2.35	Ketchum and Redfield, 1949.
Chlorella (N and P deficient culture)	55	2.4	0.9	Ketchum and Redfield, 1949.
Chlorella (N and P deficient culture)	50.9	3.0	1.1	Ketchum, 1939b.
Myxophyceae				
Typical analysis	28	4.5	0.8	Vinogradov, 1953.
Mixed phytoplankton population from Long Island Sound	20 (approx.)	3.1	0.44	Riley *et al.*, 1956.

3. NITROGEN

Like phosphorus, the nitrogen content of algae can vary greatly according to the amount of the element that is present in the medium from which the plants are grown. Deficient cells, when resupplied with nitrogen, can make up their deficiency in the dark or light, but more rapidly in the light (cf. Ketchum, 1939a; 1939b; Ketchum and Redfield, 1949; Harvey, 1953a; Thomas and Krauss, 1955).

The C:N ratio was reported by Redfield (1934) to be between about 6:1 and 8:1 in diatoms and probably near to 7.5:1 in dinoflagellates; von Brand (1935) finds 6.3:1 for *Skeletonema costatum*. Other values can be estimated from data such as given in Table I (see Section I).

4. CARBON

Some carbon values are also given in Table I. Ryther (1956a) has calculated carbon and hydrogen percentages on an ash-free organic matter basis and these elements are then seen to be present in remarkably constant amounts (45-55% for carbon and near to 7% for hydrogen). Oxygen varies considerably however (20-45%), as the amount of this element reflects the variation of fat, protein and carbohydrate in the plants, a variation which depends a lot upon the physiological condition of the phytoplankton.

B. MAJOR MOLECULAR CONSTITUENTS

A knowledge of the molecular composition of phytoplankton is perhaps not essential for the measurement of standing crop or productivity but it is necessary if one wishes to assess the nutrient or calorific value of an algae crop. In theory at least, the primary product could be measured by one of the principal molecular constituents, if such a measurement were sufficiently simple and speedy (see later, in Section III).

The data in the literature for the molecular composition of marine phytoplankton are relatively sparse, although a considerable amount of information has accumulated for freshwater plankton and for sessile forms of algae, and this information may be of some general applicability if used with caution (see reviews by Heilbron, 1942; Black, 1953; and the monograph by Fogg, 1953).

The saturated hydrocarbon, hentriacontane, $C_{31}H_{64}$, has been reported in diatoms and other algae (Heilbron, Parry and Phipers, 1935; Clarke and Mazur, 1941) and there is a range of photosynthetic pigments present (see later) although there is apparently little or no vitamin A in phytoplankton (Gillam *et al.*, 1939). Other growth factors, B vitamins, etc., (except for B_{12}, sterols and some plant hormones) do not appear to have been identified directly in marine phytoplankton but they are present in many algae (ref. Black, 1953; Ericson and Carlson, 1953). Such substances must be synthesized to some extent by any plant cell capable of truly autotrophic growth.

It is general practice to classify molecular constituents, other than pigments, under the headings of carbohydrates, lipids and proteins. The amounts of the latter have generally been estimated from Kjeldahl nitrogen and a factor of about 6.25, which has a conventional rather than a real significance. Separation by solution in 90% formic acid has also been used (Mazur and Clarke, 1942). Prolonged ether or acetone extractions of the dried material separate a "lipid" fraction, which can be further classified by saponification, etc., and, as often as not, the remaining substances, except for ash, are termed 'carbohydrates' (ref., for example, Birge and Juday, 1934; Gillam *et al.*, 1939; Clarke and Mazur, 1941; and Spoehr and Milner, 1949). Although this approach is adequate for many purposes, more refined and direct techniques are now available and should be used whenever practicable.

An idealized evaluation of carbohydrate, lipid and protein was made by Spoehr and Milner (1949) using only the ultimate analyses for carbon, oxygen, hydrogen and nitrogen. These elements were then apportioned into the three molecular classifications by means of "representative" formulae (see also Krogh *et al.*, 1930).

The Bacillariophyceae have cell walls consisting of a pectin-like substance (apart from the hydrated silica present) but accommodate most of their reserve food storage in the form of fat which is often conspicuous as "oil drops". In these respects the Xanthophyceae and Chrysophyceae somewhat resemble the Bacillariophyceae. The Dinophyceae, on the other hand, generally have cell walls of cellulose and store reserve food as starch. The planktonic Chlorophyceae, likewise, have normally a cellulose covering and store starch, although fat is found to some extent in both the last two classes. The cell walls of the Myxophyceae are composed of either pectin or cellulose with a starch-like substance acting as food reserve (eg. Fritsch, 1935, 1945; Cupp, 1943; Fogg, 1953; and other references cited therein.)

1. CARBOHYDRATES

Carbohydrate formation appears to be greatest in algae growing under slightly deficient conditions of nutrition and at a moderately high light intensity, during the late stages of exponential growth (ref. Section V). Both phosphorus and nitrogen deficiency, when serious, causes a decrease in the carbohydrate content of cells but the effect is most pronounced with a deficiency of phosphorus. (ref. Pearsall and Loose, 1937; Myers and Cramer, 1948; Spoehr and Milner, 1949; Ketchum and Redfield, 1949; Fogg, 1956). As cultures age they tend to produce more oligo- and polysaccharides. Some values for total carbohydrate are collected in Table II.

Glucose and other reducing sugars (both 5- and 6-carbon atom) have been reported in diatoms but not in very significant amounts (Barker, 1935; Bidwell *et al.*, 1952; Barashkov, 1956) and, similarly, they are absent or nearly absent from the Myxophyceae (Payen, 1938). Low concentrations are reported in the Chlorophyceae (Pearsall and Loose, 1937). Despite the importance of mannitol in the Phaeophyceae the present writer has not seen it reported in any phytoplankton other than the Xanthophyceae (Bidwell, 1957). Ascorbic acid certainly occurs in the Chlorophyceae and probably in most other algae, there being some 2-3% in *Chlorella* (ref. Black, 1953; Rabinowitch, 1956).

Oligosaccharides of two or three units have been reported in diatoms (Barashkov, 1956) and occur (sucrose) in the Chlorophyceae and (trehalose) in the Myxophyceae (ref. Fogg, 1953).

Glucose polymers form the main carbohydrate reserve of the Chlorophyceae, Dinophyceae and Myxophyceae. The compounds are starch-like in their iodine reactions and are mainly amylose and amylopectin in nature but some are less easily characterized, such as the cyanophycean starch of the Myxophyceae (Payen, 1938; Kylin, 1943; Hutchens, Podolsky and Morales, 1948; Blinks, 1951; Bidwell *et al.*, 1952; Hough, Jones and Wadman, 1952).

Lewin (1955a) has now shown that the "pectin" layer in the cell walls of a diatom is a polyuronide consisting solely of glucuronic acid residues and this polyuronide can constitute from 20 to 50% of the dry weight of *Navicula pelliculosa*, increasing with the age of the culture. Although "pectin" also occurs as a cell wall constituent of the Chlorophyceae and Dinophyceae the envelopes of these organisms are normally composed of cellulose and cellulose occurs sporadically in the Myxophyceae (ref. Fogg. 1953).

2. Lipids

The lipid content of algae varies from class to class. Some, such as the Myxophyceae, have a low lipid content. Others, in particular the Bacillariophyceae, contain amounts up to 20 to 30% of the dry ash-free organic matter, but the amount is very dependent on growth conditions. Nitrogen deficiency and growth at very high light intensity is well known as a cause of high fat content and old starved cells seem capable of changing carbohydrate to fat long after cell division and photosynthesis have slowed down or ceased (Spoehr and Milner, 1949; Ketchum and Redfield, 1949; Fogg, 1956). The total lipid content of several algae is reported in Table II.

TABLE II. Some analytical data for "Carbohydrate", "Lipid" and "Protein" in phytoplankton. (Percentages in dry material.)

Phytoplankter	Carbohydrate	Lipid	Protein	Reference
	%	%	%	
Bacillariophyceae				
Navicula pelliculosa	...	7.5	...	Low, 1955.
Nitzschia linearis	...	10.8	...	Low, 1955.
Nitzschia closterium	...	5.0	...	Low, 1955.
Nitzschia closterium (ash free) (20% ash)	33	25	42	Ketchum and Redfield, 1949. (Much lower ash content than reported by Low, 1955.)
Phaeodactylum tricornutum (ash free) (13% ash)	22	28	39	Lewin *et al.*, 1958
Mixed	13	15.5	15.5	Serenkov and Barashkov, 1934.
Mixed	16	9	61	Kunne, quoted by Laevastu, 1957.
Mixed (mean figures)	15	6	36	Brandt and Raben, 1920.
Dinophyceae				
Mixed Peridineae	22	4	50	Brandt and Raben, 1920.
Mixed Peridineae	18.5	6	67	Kunne, quoted by Laevastu, 1957.
Myxophyceae	...	4	...	Mazur and Clarke, 1942.
Chlorophyceae				
Chlorella pyrenoidosa	32	18	50	Spoehr and Milner, 1949.
Chlorella pyrenoidosa	27	25	48	Ketchum and Redfield, 1949.
Chlorella vulgaris	32	20	48	Ketchum and Redfield, 1949.
Scenedesmus obliquus	23	26.5	50	Ketchum and Redfield, 1949.
Scenedesmus obliquus	41.5	13.5	5.75	Krogh *et al.*, 1930
Stichococcus bacillaris	38	21	41	Ketchum and Redfield, 1949.
Chlorella (N deficient medium)	6	85	9	Spoehr and Milner, 1949.
Chlorella (N deficient medium)	45	46	15.5	Ketchum and Redfield, 1949.
Chlorella (P deficient medium)	22.5	24	53	Ketchum and Redfield, 1949.
Chlorella (N and P deficient medium)	38–57	23–44	16–33	Ketchum and Redfield, 1949.
Mixed freshwater population	34.6	20.3	13.8	Krogh *et al.*, 1930.

The content of *free* fatty acids is quite high in many phytoplankters and, although this has been attributed partially to the treatment of samples before analysis (Mazur and Clarke, 1942), the exceptionally high free acid values for the diatoms have been shown to be independent of any preliminary treatment, or of the biological composition of the samples. Up to 80% of the total acids in the Bacillariophyceae may be in an unesterified form and, contrary to the findings for most animal and vegetable oils, this percentage *decreases* with the increasing age of a sample so that, after storing for 6 months or so under aerobic or anaerobic conditions, marine diatoms show a decrease in free acid content and an increase in saponifiable matter (Clarke and Mazur, 1941).

The lipid composition of fresh water and marine phytoplankton is very similar to that of the animals that graze it. The bulk of the saturated acids consist of palmitic and stearic acids (in about a 2:1 ratio). Unsaturated acids generally predominate, however, and these have been separated by methyl ester distillation and shown to range from C_{14} to C_{30} members, mainly those with 16, 18 and 20 carbon atoms (Lovern, 1936; Clarke and Mazur, 1941; Kelly *et al.*, 1958).

Apart from some rather ill-defined fractions classified as "alcohols", the remaining lipid material of interest consists of various sterols, which occur to the extent of a few tenths of a per cent of the dry weight of some algae. Sterols similar to the sitosterols of higher plants were reported in diatoms (Clarke and Mazur, 1941). Chondrillasterol has been tentatively identified in *Navicula pelliculosa* and occurs to the exclusion of the other sterols in *Scenedesmus obliquus* but is not present in either fresh or salt water *Nitzschia*. Fucosterol, although found extensively in the brown algae, does not appear to exist to an appreciable extent in diatoms. *Chlorella* is interesting in that it contains ergosterol (normally found only in fungi) but this sterol has not been reported in marine phytoplankton. Sterols appear to be absent from the Myxophyceae (Carter, Heilbron and Lythgoe, 1939; Clarke and Mazur, 1941; Mazur and Clarke, 1942; Fogg, 1953; Low, 1955).

Antirachitic properties are found in *Sargasum* and *Fucus* floating on or near the sea surface. Phytoplankton oils from coastal areas may contain the D_3 provitamine, but only in the open ocean is the water of sufficient clarity for significant short wavelength activation to be possible (Johnson and Levring, 1947; Jerlov, 1951).

3. Protein, Amino Acids and Other Nitrogenous Compounds

The protein content of the algae is a direct function of the amount of available nitrogen in the growth medium. It is quite high in cells during vigorous exponential growth at a low light intensity and the algae constitute a primary and secondary source of high protein diet which could be of some value (cf. Hundley *et al.*, 1956). The protein content decreases to a fifth or less of its normal value when the growth of cells is retarded due to nitrogen deficiency.

On replenishing nitrogen in the external medium there is a high rate of re-assimilation (Pearsall and Loose, 1937; Ketchum, 1939b; Spoehr and Milner, 1949; Myers and Cramer, 1948; Thomas and Krauss, 1955). Once an algal cell has stored protein, however, the amount present remains remarkably constant in the face of any subsequent adverse conditions of nutrition or light, provided that the nitrate or ammonia level in the environment is maintained. The protein nitrogen was retained tenaciously by cells of *Scenedesmus* even after a dark period of several weeks (Krauss, 1956).

Except for the chromo-proteins, discussed later, little or no work has been reported on phytoplankton proteins, as such. Water-soluble peptides are known to be present in the Chlorophyceae and Myxophyceae, the latter class of algae excreting considerable amounts of extra-cellular polypeptides (Fogg, 1952; Fogg and Wolfe, 1954; Fogg and Westlake, 1955). Analyses of *Scenedesmus* (ref. Krauss, 1956) show that about 93% of the nitrogen is present as protein N, about 3% as peptide and free amino acid N, and the remaining 4% as various forms of low-molecular-weight soluble compounds.

Nucleoproteins and nucleic acids must obviously occur in the algae but, as with plants in general, the subject has received comparatively little attention. By analogy with terrestial plants, it is likely that some 20% or more of the phosphorus in algae is combined as nucleoprotein, apart from the sizeable concentrations of nucleotides that must also be present (ref. Chargaff and Davidson, 1955; Arnon, 1956). There appears to be several per cent of RNA and DNA in vigorously growing Chlorophyceae and Bacillariophyceae, especially at the end of exponential growth (Jeener, 1952a, b; Curl, 1957). What is probably polynucleotidal material is excreted by phytoplankton (Bowen, 1957). Low (1958) has reported on the purine and pyrimidine ratios in several phytoplankters.

Most of the data in the literature are confined to benthic algae and to the amino acid analysis of the hydrolysate of bulk protein. Earlier attempts to show differentiation between algal classes, based on the amino acid spectrum of individuals, are now generally disputed and it is most probable that there is a general similarity between the amino acid composition of all algae and higher plants. There is a surprisingly good spectrum of "essential nutrient" amino acids, except perhaps for cystine and threonine (see review and bibliography by Hundley *et al.*, 1956, and the review and extremely detailed paper by Channing and Young, 1953; also Lugg, 1949, and Ericson and Carlson, 1953). Of the essential acids in *Chlorella*, however, only valine, leucine and isoleucine are present in quantity; alanine and glutamic acid are the most abundant constituents (Eny, 1949). The Myxophyceae are characterized by the presence of α-ε-diaminopimelic acid, which occurs elsewhere only in certain bacteria (ref. Fogg, 1953).

Low (1955) has shown by two-dimensional chromatography that there is no qualitative difference in the amino acid make-up amongst the genera of either fresh water or marine diatoms. All 19 common amino acids were present but the presence of citrulline and hydroxyproline was doubtful. Takagi (1953) and Ericson and Carlson (1953) report fully on the serine, threonine, tyrosine tryptophan and other amino acid contents of various marine algae. Takagi (1956) has also shown that glycine, alanine, serine, valine, leucine, phenylalanine and aspartic and glutamic acids, (especially valine, leucine and phenylalanine) are particularly abundant in several classes of marine algae but he did not detect tyrosine, histidine or lysine, and threonine, cystine, methionine and proline were not abundant. Glycine, alanine, serine, threonine, proline, aspartic acid and glutamic acid were found in the *free form*. Recent work (Linko *et al.*, 1957) has indicated that the Myxophyceae build up citrulline in significant quantities at an early stage in photosynthesis. This acid probably acts as a storage pool for the carbamyl group or as a route for ammonia in protein synthesis. There may be a variation of protein composition according to the trace metal deficiencies of the growth media but as yet this has been shown only with a higher plant (Schutte and Schendel, 1958).

C. PIGMENTS

The plant pigment content of phytoplankton assumes considerable importance in productivity studies because of the use of these compounds for estimating the primary product and gross phytosynthetic potential (ref. Sections III.E and IV.G). The meagre amount of *quantitative* information that is available concerning the pigment content of planktonic algae has already been discussed in Section I.B. The purpose of the present section is to outline some of the qualitative data that is of particular interest in productivity measurement studies.

Reviews of the algal pigments have been given by Carter *et al.* (1939), Heilbron (1942), Cook (1945), and Strain (1951) amongst others. The similar distribution of certain pigments in algae of a given Class (or even Division) suggests that pigments developed before many orders or genera (Strain, 1951) and that they might possibly be used for semi-quantitative taxonomic sortings. The pigments present in the four classes of algae that comprise the bulk of the marine phytoplankton are summarized by Table III. The arrangement is similar to that given by Strain (1951) with data from Seybold and Egle (1938), Tisher (1938), Carter *et al.* (1939), Pace (1941), Strain and Manning (1942a), Gardiner (1943), Strain, Manning and Hardin (1943 and 1944), Strain (1951), Goodwin (1957) and other sources. The Chrysophyceae appear to resemble the Bacillariophyceae rather closely in their pigmentation; little is known concerning the Xanthophyceae.

The chlorophyll pigments produced both in the dark and the light appear to be identical (Meyers, 1940) but the rate of production tends to increase during hours of low illumination. Conversely in very strong light, chlorophylls are destroyed by the well-known photoxidation reaction. The chlorophyll content of a cell is also a direct function of the physiological health of the cell, as governed by nutrition and age, and decreases markedly to give a chlorotic condition to algae grown in a medium deficient in phosphorus, nitrogen, magnesium or iron (ref. for example, Emerson, 1929; Riley, 1939a; Haskins, 1941; Harvey, 1953a; Ryther, 1954a; Marshall, 1956; Yentsch and Vaccaro, 1958). The carotenoid pigments are reputed to undergo similar variations in concentration according to nutrition but are generally less sensitive to nutrient deficiencies and to light than are the chlorophylls (Haskin, 1941; Ketchum, 1957; Ryther *et al.*, 1958; Yentsch and Vaccaro, 1958). A combination of bright light and nitrogen deficiency can change the chlorophyll: carotenoid ratio in *Chlorella* from 8.5:1 to 0.7:1 (Aach, 1953). Similar ratio changes were recorded in a number of marine algae by Yentsch and Vaccaro (1958). Margalef (1954) found that the chlorophyll content per cell of *Nitzschia closterium* decreased with decreasing temperature.

1. THE CHLOROPHYLLS AND RELATED PIGMENTS

The separation and purification of chlorophylls from each other and from other pigments is invariably carried out by chromatographic techniques that are fully described in most papers dealing with the preparation of plant pigments (for example Richards, 1952; Strain, 1946). The separation of chlorophyll *c* from the last traces of chlorophyll *a* presents the greatest difficulty and has probably not yet been achieved. Chlorophylls can be extracted from plant material by most polar organic solvents when these solvents have some water added to assist in the severance of pigment-protein bonds in the chloroplasts.

It will be seen from Table III that chlorophyll *a* is the predominant chlorophyll pigment in planktonic algae, as it is in terrestial plants. Early suggestions that chlorophyll *b* (an important chlorophyll in terrestial botany) was also present in diatoms (cf. Kylin, 1927; Pace, 1941) have now been conclusively disproved and the presence of this pigment has been substantiated only with the Chlorophyceae (Seybold and Egle, 1938; Dutton and Manning, 1941; Gardiner, 1943; Richards, 1952). It is thus of relatively little importance in marine studies. On the other hand, chlorophyll *c*, a pale yellow-green pigment earlier known as chlorofucine or chlorophyll γ, is found in several of the planktonic algae. It is present in all diatoms and especially in the photosynthetic dinoflagellates (Strain and Manning, 1942a; Strain, Manning and Harding, 1943; Richards, 1952). Although visually not an impressive pigment the absorption bands in its spectrum that are located at 4700 A and 5850 A, especially the former, are quite effective in capturing the more penetrating wavelengths of submarine radiation and chlorophyll *c* may have a greater significance in marine productivity studies than was first realized.

The "Fisher" structures for chlorophylls *a* and *b* are now well established as derivatives of dihydroporphyrin containing a central non-ionizable magnesium atom. In addition to the four pyrrole nuclei surrounding the magnesium there is a cyclopentanone ring with a methyl ester group attached. A propionic acid

Table III. Pigments in the phytoplankton.

Pigment	Bacillario-phyceae	Dinophyceae	Chloro-phyceae	Myxophyceae
Chlorophylls				
Chlorophyll *a*	xxx	xxx	xxx	xxx
Chlorophyll *b*	—	—	xx	—
Chlorophyll *c*	xx	xx	—	—
Carotenes				
α-Carotene	—	—	x	—
β-Carotene	xxx	xxx	xxx	xxx
Xanthophylls				
Zeaxanthin	—	—	x?	x
Lutein	—	—	xxx	x
Violaxanthin	—	—	x	?
Fucoxanthin	xx	—	—	?
Neofucoxanthin (A and B)	x	—	—	?
Diatoxanthin	x	—	—	?
Diadinoxanthin	x	x	—	?
Neodiadinoxanthin	x?	x	—	—
Dinoxanthin	—	x	—	—
Neodinoxanthin	—	x	—	—
Peridinin	—	xx	—	—
Myxoxanthin	—	—	—	xx
Myxoxanthophyll	—	—	—	xx
Echinenone	—	—	—	xx
Phycobilins				
Phycoerythrin	—	—	—	x
Phycocyanin	—	—	—	xxx

— Absent.
x Detected.
xx Present as a minor pigment within the group.
xxx Present as a major pigment within the group.

side chain, esterified by phytol (a mono-unsaturated long-chain primary alcohol), is attached to one of the pyrrole nuclei. The molecular weights of chlorophylls *a* and *b* are 893 and 907, respectively, the molecules differing from each other only by the substitution of an aldehyde group for a methyl group. The structure of chlorophyll *c* is as yet undetermined but it is known to be a porphyrin with a very strongly bound magnesium atom and a cyclopentanone ring. There is, apparently, no phytol. It is not impossible that this pigment may have some role as a chlorophyll precursor (Granick, 1949), but the position is not clear and it is now thought to be very unlikely (J. H. C. Smith, 1959, private communication).

The chlorophylls are characterized by two main absorption maxima, one in the blue and one in the red region of the visible spectrum. These bands are generally of unequal intensity, the "blue" maximum being greater than the "red" maximum. Other absorption bands occur, and there is a minimum close to 5000 A, but these are of secondary importance, and will not be considered further.

The fluorescence of chlorophyll solutions, which occurs in most polar solvents at a slightly longer wavelength than the red maxima, does not appear to have had much analytical application, although the fluorescence of plankton pigment extracts in ultraviolet light has been used for the estimation of crop (Kalle, 1951).

Both the position and intensity of the blue and red absorption maxima depend upon the solvent in which the chlorophyll is dissolved. In general, shifts in the blue maxima are irregular but the red maxima tend to move to longer wavelengths in solvents of increasing refractive index. Of some considerable practical importance is the very marked changes in intensity and wavelength that occur when even traces of polar substances are present as impurities in non-polar solvents. The use of aqueous polar solvents, like wet alcohols or ketones, greatly minimizes spectral shifts.

Many of the available data of possible analytical interest are summarized by Table IV. The reader is also referred to the excellent account by J. H. C. Smith and others (1955). Quite notable discrepancies occur in the literature due to variability in the purity of pigments and solvents and the quality of the spectrographic equipment used and it is sometimes difficult to know which values are the most reliable (Zscheile and Comar, 1941). Molecular or specific absorption coefficients are not reported for all solvents, although relative peak heights are easy to obtain. *In vivo* spectra are particularly difficult to determine with precision. The chlorophyll in individual chloroplasts, in which it may be present at 'concentrations' of the order of 0.1 M, (or some 8% to 10%) is linked to protein and probably also to lipid material. This association produces a "red shift" to longer wavelengths of some 50-100 A for the blue maximum and up to 200 A for the red maximum, compared with solutions in ether. The shift is not the same in all algae and plants. French and Elliot (1958), using differential spectrophotometry, have shown the shift to be very marked in an "old" culture of *Euglena*, the peak moving to 6950 A!

For greater detail concerning the spectral characteristics of the chlorophylls and the underlying electronic transitions, etc. the reader is referred to Rabinowitch (1951). Data for Table IV were derived from several sources, notably Zscheile and Comar (1941), Strain and Manning (1942a), Harris and Zscheile (1943), Strain, Manning and Hardin (1943), Koski and Smith (1948), Tanada

TABLE IV. Some spectral characteristics of chlorophylls and related substances.

λ = Wavelength of peak absorption in Angstrom units.

R = Ratio: $\frac{\text{extinction of blue maximum.}}{\text{extinction of red maximum}}$

E^{M}_{1cm} = Molecular extinction coefficient.

$E^{1\%}_{1cm}$ = Extinction coefficient for a 1% solution.

Solvent	Data	Chlorophyll *a*		Chlorophyll *b*		Chlorophyll *c*			Protochlorophyll *a*			Phaeo-Pigments		
		Red	Blue	Red	Blue	Red	Orange	Blue	Red	Orange	Blue	Red	Orange	Blue
Ethyl ether	λ	6600	4290	6425	4530		...		6230	5710	4320	**6680	**5050	**4100
	R	1.33		3.00			...			8.15			...	
	E^{M}_{1cm}	$9.1x10^4$	$12.0x10^4$	$5.15x10^4$	$15.5x10^4$		...		$3.28x10^4$		$26.7x10^4$		...	
Acetone	λ	6615	4315	6435	4560	6310	5810	4450	6230		4320	*6600	*5050	*4100
	R	1.26		2.95			8.0			7.75			...	
	E^{M}_{1cm}	$6.35x10^4$	$8.0x10^4$	*ca.* $4x10^4$	*ca.* $12x10^4$		...		$3.1x10^4$		$24x10^4$		...	
	$E^{1\%}_{1cm}$	710	895	*ca.* 440	*ca.* 1300		...		350		2700		...	
Methanol	λ	6640	4340	6510	4710	6300	5850	4500	6290		4340	*6525	...	*4180
	R	1.00		2.85			8.2			6.5			*4.6	
	$E^{1\%}_{1cm}$	...		...			...		270		1770	*327	...	*1490
Carbon Tetrachloride	λ	6635	4325	6440	4570		...			...			...	
	R	1.3		2.54			...			...			...	
In vivo	λ	*ca.* 6750 to 6950	*ca.* 4400	*ca.* 6500	*ca.* 4650	*ca.* 6400	*ca.* 5850	*ca.* 4700	*ca.* 6500				...	

*Phaeophytin *a* **Phaeophorbide *a*

(1951), Richards (1952), Livingstone *et al.* (1953), Röbbelen (1956), Yentsch (1957) and values collected by Rabinowitch (1951 and 1956).

Of the chlorophyll precursor pigments, the protochlorophylls, protochlorophyll *a* is probably the only one that need concern us. It is an oxidized form of the parent molecule and differs from it only by two hydrogen atoms in the pyrrole nucleus containing the phytol. This substance accumulates in the dark and most of it is converted to chlorophyll on illumination. The absorption spectrum of protochlorophyll *a* is not unlike that of chlorophyll *c* and values for the latter might easily be in error if significant amounts of protochlorphyll *a* are present. The writer knows of no study in which this has been considered and the presence of a protochlorophyll in algae does not appear to have been confirmed.

Chlorophyll, in solution in alcohols or acetone, can be oxidized by many mild oxidants, including air, and is said to be "allomerized". The blue maximum is reduced but there is little change in the red maximum except for a slight displacement to shorter wavelengths. This behaviour is not found with ether or non-polar solvents. In all solvents, however, a slow bleaching of chlorophyll solutions will take place, especially in the light (photoxidation). The effect is less with crude plant extracts than with pure chlorophyll solutions and the former are generally stable for several days if kept in the dark at temperatures below 0-5° C.

It is essential, however, that solutions are prevented from becoming acid (pH less than about 5) at any stage during the extraction, separation, or storage of chlorophylls. This is generally ensured by the addition of a weak organic or inorganic base (e.g. dimethylaniline or magnesium carbonate) to the solvent. If acid is present "pheophytinization" occurs, magnesium being eliminated from the chlorophyll molecule and replaced by hydrogen, a process accelerated by light. Pheophytin *a*, the only pheophytin likely to be produced in quantity by marine phytoplankton, is olive-green and has a very similar absorption spectrum to chlorophyll *a* in the red, except that the band is greatly weakened (Livingstone *et al.*, 1953). A small peak at 5050 A (see Table IV), however, is not present in any of the chlorophylls and might be used for the identification of pheophytin were carotenes and xanthophylls first removed, otherwise the presence of pheophytin *a* in detritus will go undetected by most methods (see later) and can give rise to significant errors.

Elimination of phytol from pheophytin *a* by the enzyme chlorophyllase, occurring in plant tissue, gives rise to pheophorbide *a*. This compound also has an absorption peak at about 5050 A but otherwise its spectrum is reminiscent of chlorophyll *a*, with a weakened red band (ref. Table IV).

The possible decomposition reactions mentioned above occur to an accelerated extent in dead plant cells, unless these are rapidly *dried* and stored in a desiccated condition in the dark at low temperatures. With such precautions the pigments can be preserved unchanged for many weeks or months. It might be advantageous to store samples in an inert atmosphere (see for example, Seybold and Egle, 1938; Zscheile *et al.*, 1944; Creitz and Richards, 1955).

2. THE CAROTENES

The distribution of the carotenes in the four main classes of marine algae is shown in Table III. Only β-carotene is reported in the Chrysophyceae and Xanthophyceae (Strain, 1951) and appears to resemble chlorophyll *a* in being an essential pigment in all marine algae. A review of the chemistry and structure of the carotenes is given by Heilbron and Cook (1951) and their isolation, purification and identification in algae is described in many papers (ref. Heilbron and Lythgoe, 1936; Seybold and Egle, 1938; Tisher, 1938; Pace, 1941; Haagen-Smit *et al.*, 1943; Strain, Manning and Hardin, 1944). The separation of α-carotene from β-carotene is described fully by Strain (1939).

The carotenes are yellow-orange fat-soluble unsaturated hydrocarbons with 40 carbon atoms and a large number of conjugated double bonds. They appear to be exclusively plant pigments. Despite many possibilities of geometrical isomerism only the trans isomers are reported in nature. The β compound has 11 double bonds, all conjugated. In α-carotene one bond is shifted into a terminal ionone ring. Both these carotenes have a molecular weight of 536. The unusual

TABLE V. Some spectral characteristics of α- and β-carotene.

Solvent	Data	β-Carotene		α-Carotene	
		Band I	Band II	Band I	Band II
Chloroform	λ	4970	4660	...	...
Petroleum ether	λ	4800	4500	4740	4440
	$E_{1\,cm}^{M}$	12.0×10^4	13.5×10^4	13.8×10^4	15.1×10^4
	$E_{1\,cm}^{1\%}$	2240	2520	2575	2820
Acetone	λ	4800	4540	...	...
	$E_{1\,cm}^{M}$	11.95×10^4	13.45×10^4	...	...
	$E_{1\,cm}^{1\%}$	2230	2510	...	...
Hexane	λ	4790	4500	4740	4440
	$E_{1\,cm}^{M}$	12.2×10^4	13.9×10^4	13.4×10^4	14.7×10^4
	$E_{1\,cm}^{1\%}$	2280	2590	2500	2750
Ethyl ether	λ	4780	4490	...	...

λ = Wavelength of peak absorption in Angstrom units.
$E_{1\,cm}^{M}$ = Molecular extinction coefficient.
$E_{1\,cm}^{1\%}$ = Extinction coefficient for a 1% solution.

and rare ε-carotene has been reported only in the one diatom *Navicula torquatum* (Strain and Manning, 1943; Strain *et al.*, 1944).

There is little or no fluorescence reported for the carotenes *in vivo* or in solution. The absorption spectra show a characteristic band in the ultraviolet and near visible region of the spectrum, from about 3250 A to 5000 A, with a "shoulder" near to 4300 A and two broad peaks. A few values for the position and magnitude of the absorption maxima in various solvents are collected in Table V, using the data of Strain (1939), Zechmeister and Polgar (1943), Richards (1952) and values quoted by Rabinowitch (1951). Nearly all quantitative values in the literature are for solutions in hydrocarbon but Richards (1952) obtained acetone data in connection with the development of analytical techniques. As with the chlorophylls, peak shifts are towards longer wavelengths with increasing polarity and polarizability of solvent. The carotenes, *in vivo*, are associated with lipids and proteins which give rise to a "red shift" in their spectra the exact magnitude of which is difficult to estimate but which is known to be considerable, approaching 200 A or more (Dutton and Manning, 1941; Tanada, 1951).

α- and β-carotene solutions are quickly bleached by light and undergo oxidation, cis-trans changes and other transformations, especially when acid is present. The deterioration of these pigments is even greater whilst they are still present in plant tissue and precautions during storage and solvent extraction, similar to those already described for the chlorophylls, are essential.

3. THE XANTHOPHYLLS

The rather complex distribution of the xanthophyll pigments, the third major group of pigments in marine planktonic algae, is summarized in Table III. These pigments are the most important carotenoids in that their combined weight (except in the Myxophyceae) generally exceeds that of the carotenes by a factor of some 2 to 8 times. The xanthophylls and carotenes together, however, rarely amount to a quarter of the quantity of chlorophyll present in algae, unless the plants are severely chlorotic due to nutrient deficiences. In this case the total weight of carotenoid pigments may approach that of the chlorophylls (Seybold and Egle, 1938; Gillam *et al.*, 1939; Pace, 1941; Haskin, 1941 and 1942; Aach, 1953; Ketchum, 1957; and Yentsch and Vaccaro, 1958). It should be realized that the reporting of a particular xanthophyll in a particular class of algae in Table III does not imply that the pigment is necessarily present in all genera of this class.

The well-known chromatic adaption of algae (ref. for example Stanbury, 1931) generally affects the chlorophyll: carotenoid ratio but Strain *et al.* (1944) have reported a marked response within the xanthophyll group (an increase of diadinoxanthin in red light). It is even possible that new xanthophylls may form as a result of nutrient deficiencies (Haskin, 1941). It is not known how general this effect may be nor what significance it may have, if any, for marine productivity studies. Pigment changes are not always of necessity in the direction of chromatic adaptation (Yokum and Blinks, 1958).

For an account of the general chemistry and properties of the xanthophyll pigments the reader is referred to the monographs by Strain (1938, 1944), the study by Carter *et al.* (1939) and the excellent paper by Strain *et al.* (1944). In the latter will be found a full account of the chromatographic behaviour of many of the marine plant xanthophylls on magnesia and sugar columns.

The xanthophylls are closely related to the carotenes, being C_{40} compounds with some 10 to 12 conjugated double bonds joining ionone rings, but they also contain oxygen atoms (from 1 to 7 or 8) present in hydroxyl or carbonyl groups. Most naturally occurring isomers are trans (cf. β-carotene) but less stable cis-isomers have been reported and are generally designated by the prefix "neo-" (Strain and Manning, 1942b), although it should be mentioned that such compounds have been suspected of being oxidation products (Heilbron *et al.*, 1935; Kylin, 1939). The structure of some xanthophylls still remains to be elucidated. The terrestially abundant lutein ("luteol" or "xanthophyll") is known to be a dihydroxy α-carotene. Fucoxanthin contains 6 oxygen atoms per molecule, as 4 hydroxyl groups and 2 carbonyl groups on broken terminal ionone rings (Heilbron *et al.*, 1935); and the pigment, myxoxanthophyll, has as many as 7 oxygen atoms, although myxoanthin has only one (ref. Heilbron and Lythgoe, 1936). The molecular weight of all these compounds lies between about 550 and 600.

The Chlorophyceae resemble terrestial plants, of which they were probably the remote ancestors (Smith quoted by Strain, 1951), in having a high content of lutein and pigments such as violaxanthin and zeaxanthin. Among the Chrysophyta we find that the Xanthophyceae are very rich in xanthophylls, from which most of them derive their yellow-green colour, although the nature of these pigments has received little study. Flavoxanthin has been reported from one species. The golden-yellow to brown Bacillariophyceae and Chrysophyceae probably have a very similar xanthophyll spectrum but the Chrysophyceae have not been examined in much detail. In the diatoms, lutein appears to be absent and its place taken by fucoxanthins and the specific diatoxanthin. In the Dinophyceae fucoxanthin is absent and its role is taken by the similar pigment peridinin and the specific dinoxanthin. Diadinoxanthin, with a spectrum almost identical with that of lutein, occurs, as its name implies, in both diatoms and dinoflagellates. Although both lutein and zeaxanthin have been reported in the Myxophyceae, the main xanthophylls of the blue-green algae are the specific myxoanthin and myxoxanthophyll pigments, the former resembling the crustacean pigment, astacin. Echinenone, an animal pigment, and a large number of minor pigments are also reported (Goodwin, 1957).

There is little or no fluorescence of xanthophylls in solution or *in vivo*. Superficially the absorption spectra of these pigments resemble those of the carotenes, with two peaks in about the same region. In polar solvents the two maxima sometimes give way to one very broad maximum, extending over several hundred Angstrom units. The solvent "red shifts" are very similar to those found with the chlorophylls and carotenes. The spectra of many xanthophylls

TABLE VI. Some spectral characteristics of the xanthophylls.

Pigment	Solvent	Wavelength of maximum absorption	
		Band I	Band II
Lutein	Chloroform	4850	4570
	Ethyl alcohol	4730	4510
	Ethyl ether	4720	4470
Violaxanthin	Ethyl alcohol	4720	4420 and 4170
Fucoxanthin	Acetone	4850	4490
	Chloroform	4840	4590
	Petroleum ether	4780	4510
	Methyl alcohol	4760	4500
	Ethyl alcohol	Broad band at 4530 approximately	
Neofucoxanthin A	Acetone	Broad band at 4480 approximately	
	Ethyl alcohol	Broad band at 4470 approximately	
Neofucoxanthin B	Acetone	Broad band at 4480 approximately	
	Ethyl alcohol	Broad band at 4460 approximately	
Diatoxanthin	Ethyl alcohol	4810	4530
	Acetone	4780	4510
Diadinoxanthin	Ethyl alcohol	4780	4480
	Acetone	4720	4445
Neodiadinoxanthin	Ethyl alcohol	4710	4420
Dinoxanthin	Ethyl alcohol	4710	4415 and 4180
Neodinoxanthin	Ethyl alcohol	4660	4380
Peridinin	Petroleum ether	4880	4560
	Ethyl alcohol	Broad band at 4750 approximately	
	Acetone	Broad band at 4650 approximately	
Myxoxanthin	Chloroform	Broad band at 4730 approximately	
	Ethyl alcohol	Broad band at 4700 approximately	
	Petroleum ether	Broad band at 4650 approximately	
Myxoxanthophyll	Chloroform	5180 and 4845	4500

are almost identical (cf. zeaxanthin and diatoxanthin, diadinoxanthin and lutein, dinoxanthin and violaxanthin, etc.) and positive identification, therefore, depends upon a combination of spectral and chromatographic properties. Very few specific or molecular absorption coefficients are available but the value of E^{M}_{1} cm at peak wavelengths will be near to 12×10^4, as the oscillator strengths of these compounds should approximate to those of the carotenes. The absorption maxima for those xanthophylls likely to be of interest in marine productivity studies have been collected in Table VI, using data from Heilbron and Lythgoe (1936), Strain (1938), Tischer (1938), Carter *et al.* (1939), Strain *et al.* (1944), Wassink and Kersten (1946) and Richards (1952).

The xanthophyll pigments exist in the chloroplasts of plants combined with both protein and lipid, in some form of molecular association, which is probably more pronounced than that found with the carotenes and chlorophylls. There is, therefore, a very marked "red shift" of the absorption maxima and a broadening of spectra towards longer wavelengths as a result of this association, which gives rise to *in vivo* absorptions some 300-400 A further into the red than is found with solutions (ref. Rabinowitch, 1951). The importance of this shift for the photosynthetic efficiency of phytoplankton has been discussed by several authors (ref. e.g. the review by Strickland, 1958).

On treating phytoplankton in a manner designed to denature protein and break the pigment–protein and pigment–lipid bonds (e.g. heat, decay, formaldehyde preservation) the xanthophyll absorption spectra revert back into the blue region and many plants lose their brown colour, becoming green due to the appearance of unmasked chlorophyll.

The xanthophylls resemble the carotenes in their general instability, especially in dead plant tissue, but may be somewhat more resistant to atmospheric attack in the dark. Solutions rapidly deteriorate if exposed to strong illumination at room temperatures.

4. THE CHROMOPROTEINS

Although important pigments in the Rhodophyceae the chromoprotein pigments (phycobilins) are of little concern in studies of marine phytoplankton productivity as they are found only in the Myxophyceae. The blue-green algae will rarely be abundant except in brackish inlet waters. The phycobilin pigments are metal-free compounds in which a tetrapyrrole group (related to the bile pigments) is joined firmly to a globulin-like protein. The protein part of this molecule has been the subject of much investigation but need not concern us. Of the two types of phycobilins, the red phycoerythrins and the blue phycocyanines, the latter predominate in the blue-green algae but some species of blue-green algae may actually be red due to a preponderance of phycoerythrin.

The total chromoprotein content of these algae is probably less than the chlorophyll content, namely a few tenths of a per cent. The pigments are interconvertible by oxidation or reduction (phycocyanines are the oxidized forms) and, unlike the chlorophylls and carotenoids, they are readily water soluble to give solutions with a beautiful fluorescence. The phycoerythrins in aqueous solution show absorption maxima at about 5000 A, 5400 A and 5650 A, whereas the phycocyanines have one major peak in the visible at 6150A. The oscillator strength is high, giving an estimated E_1^M cm of 15×10^4 or greater (see Rabinowitch, 1951 and 1956). There is a slight red shift with these pigments *in vivo* when compared with aqueous extracts but the effect is very small. The phycobilins are comparatively stable in aqueous soutions, provided that the pH does not vary much outside the range 3 to 5.

SECTION III. MEASUREMENT OF STANDING CROP

A. SAMPLING PROBLEMS

Although the use of non-metallic sampling equipment is essential when studying the metabolism of phytoplankton (ref. Section IV) it is not yet clear to what extent this applies when samples are required only for crop measurement. Provided that the plants are concentrated without delay and are stored with adequate protection against deterioration it is difficult to see why the use of clean copper or iron-base sampling equipment (especially Monel metal or stainless steel) should be forbidden.

Some organisms may be so sensitive to metal ions that they disrupt, with the loss of body fluids, before they are processed and for this reason it would be preferable to use non-metallic sampling equipment if at all possible, but it remains to be proven whether, in Arctic and sub-Arctic waters at any rate, such a use is mandatory.

Three main problems arise when sampling the standing crop of phytoplankton in either lakes or in the ocean. The sample must be representative of a plant population which is distributed unevenly over a large area, the sample must be processed in such a way that all the plants are separated rapidly and completely from a very large volume of water and the plant material should, ideally, be freed from all organic matter of animal origin and from detritus.

1. The Initial Sampling Problem

Relatively little quantitative information is available concerning the horizontal and vertical distribution of marine phytoplankton (ref. e.g. Marshall and Orr, 1930; Savage and Hardy, 1935; Hardy and Gunther, 1935; Savage and Wimpenny, 1936; Hardy *et al.*, 1936; Lucas, 1940, 1941; Allen, 1941; Hart, 1942; Sargent and Walker, 1948; Lucas and Stubbings, 1948; Gilbrich, 1952; Patrick, 1952). The subject is well reviewed by Bainbridge (1957). Conditions are so varied that it is doubtful whether any information has much general applicability. In sub-tropical waters the vertical distribution is fairly uniform but normally more plants will be present in the lower parts of the euphotic zone than at the top (see Riley, 1938a, 1939b, 1941b; Riley *et al.*, 1949; Riley *et al.*, 1956; Riley, 1957; Marshall, 1956 and other references in Section III F). When dealing with the flagellates, vertical migration may be a significant factor (ref., for example, Pomeroy *et al.*, 1956; Hasle, 1954a). Horizontal aggregation due to wind forces may be marked in shallow inshore areas (cf. Verduin, 1951; Rodhe, 1957 for results in lakes). The swarming and clustering of zooplankton has its counterpart in the blooming of phytoplankton and the sampling of phytoplankton populations ought, therefore, to be amenable to the same mathematical treatments that have been used in zooplankton work, for example the application of contagious series statistics (ref. Barnes and Marshall, 1951).

The possible size of discrete phytoplankton blooms has been considered by Kierstead and Slobodkin (1953). Blooms can vary in size from a few hundred yards to many miles in diameter. They are often oval in shape with the major axis some four times the minor axis. A rough mean size would be around 10 by 40 miles (ref. Bainbridge, 1957). The quantitative aspects of spatial heterogeneity in phytoplankton populations, especially when these are markedly polymictic, is dealt with in an excellent article by Margalef (1956) who proposes the use of a "diversity index" and a population "entropy" value, borrowing from statistical thermodynamics. This quantity is at a maximum where two patches of phytoplankton, each of a different species composition, overlap.

The necessity for sampling a large volume of water is so obvious as to need no further comment. Ordinary oceanographic water sampling bottles, even when their capacity is increased to 20 litres or more (cf. Van Dorn, 1956), are inadequate for assessing marine phytoplankton crops, although they may be satisfactory for taking samples required for culturing and taxonomic work (Hasle, 1954b). Resort to net towing, such as is used in zooplankton studies, could, ideally, solve the problem, by increasing the volume of water sampled and the area covered, but authorities are now agreed that the loss of photosynthetic organisms through even the finest netting that can be conveniently towed (the No. 25 bolting silk net) is much too serious to be neglected. Data from such equipment as the Hardy Recorder (Hardy, 1936) or Gulf Sampler (Collier, 1957) give valuable information as to relative aboundance and spatial distribution of the larger phytoplankters but are in no sense quantitative.

Wood and Davis (1956), from chlorophyll measurements, found that the plant population in the water passing a 170-mesh net (No. 20 silk) was one or two orders of magnitude greater than that caught by the net. In lakes and in the sea a large fraction of a crop can escape net towing and what is retained is not necessarily representative of the whole crop, even in sub-Arctic waters, (Nielsen, 1938; Riley, 1941a; Gessner, 1944; Harvey, 1951; Verduin, 1956b; Braarud, 1957; Hanaoka, 1957; Krey, 1957 and others). The use of a net technique (ref. Harvey, 1934a) must therefore be abandoned for quantitative work. The losses will, of course, depend upon the species composition of any particular phytoplankton bloom and can be allowed for to some extent by "calibrating" the net by a more quantitative collection technique (Kleerekoper, 1953) but the constancy of any factor thus obtained would be of short duration and the method scarcely seems to warrant the considerable labour involved.

The only satisfactory solution to the problem, that can be envisaged at present, is to pump comparatively large volumes of water from the euphotic zone whilst a ship is stationary or, preferably, in motion. The pumping can be accompanied by some suitable filtration in an underwater unit lowered from the ship, as in the "Toronto Sampler" (Langford, 1953) or suction can be applied from the deck to a filter unit located at a variable depth (Hanaoka, 1957). It is probably more convenient, however, to undertake filtration on the deck of the ship itself

(Kleerekoper, 1953) and an arrangement such as that mentioned by Carritt (1956) seems ideal. Here a hose, with its end at a variable depth in the euphotic zone, connects to a pump working at such a rate that the intake velocity to the hose is equal to the velocity of the ship. Very many gallons can be taken over a period of an hour or so whilst the ship travels a distance of 5 to 10 miles, but only a small sample needs to be retained. This is taken from a by-pass, proportionating only a few per cent of the main flow, either directly through a filter or into a container on the ship's deck from which smaller aliquot samples may be withdrawn later.

Fox *et al.* (1952) describe a subsurface autofilter in which suction is generated by placing one or more filter units in the high velocity area of the venturi intake of a sampler dragged through the water. The filtered water collects in a large container which enables one to measure its volume. The volumes are relatively small, however, and it is not clear whether or not the equipment has any useful application for phytoplankton collection.

2. SEPARATION OF PLANKTON AND WATER

Only two processes for large-scale use merit consideration, centrifugation and filtration, although concentration by settling and decantation can be used for small samples when the plankton is to be studied by microscopy (see later). Concentration by floating might be feasible if the density of the sea water could be raised by a suitable addition of a non-electrolyte but the writer has seen no reference to such a method.

There seems some difference of opinion as to the efficiency of methods based on centrifugation. Clearly any phytoplankters having a specific gravity equal to or less than the surrounding aqueous medium cannot be separated (ref. e.g. Hartman, 1958), but these seem to be restricted to certain of the Myxophyceae (see Braarud, 1957), which will rarely constitute an important fraction of the crop, except in brackish inlets. The serious losses that have been reported, ranging from some 30% (Gran, 1932) to over 90% (Allen, 1919), have led to serious condemnation of the use of centrifugation (cf. Krizenecky, 1942 and Braarud, 1957) and yet, at the other extreme, centrifugation has been reported to be quite satisfactory, even for nannoplankton (e.g. Nielsen and Brand, 1934; Ballantine, 1953; Davis, 1957; Kutkühn, 1958), especially if a "collecting agent" such as aluminium hydroxide is added to the sample.

The common-sense explanation of such apparent contradictions seems to be that they result from the use of different durations and intensities of centrifugation. A sufficiently slow "pass" through a sufficiently powerful continuous through-put centrifuge (say a few liters an hour at 15,000-30,000 rpm), which will remove over 90% of most bacteria, can scarcely fail to separate a large fraction of the total phytoplankton crop, even the ultraplankton. The nannoplankters do not necessarily comprise a high fraction of the total crop in temperate waters (Cushing, 1955), except perhaps in the late summer (Margalef, 1956), and preliminary tests by pigment analyses (see later) should suffice to check the efficiency of centrifugation in any particular circumstance. In this connection it might be best to measure chlorophyll *c* or peridinin, which will give a more sensitive measure of the less easily separated ultra-Dinophyceae (Creitz and Richards, 1955). It is very probable, of course, that high speed centrifugation will disintegrate some delicate organism in the phytoplankton but much of the body substance is not necessarily lost.

Filtration through the finer analytical grade filter papers, with a mean pore size of about 2 to 4 microns, should filter out all micro and nannoplankters, especially if a double thickness of paper is used. Any losses of particulate matter are relatively small, being restricted mainly to bacteria, the smallest ultraplankters and possibly a little very fine inorganic matter (Keys *et al.*, 1935; Armstrong and Atkins, 1950; Harvey, 1950). The standard filter for the quantitative retention of phytoplankton, however, is now the collodion membrane filter. Such membranes have a uniform pore size and have some 80% of their surface area freely permeable to liquid, making for very rapid filtration. The Millipore Corporation H.A. filter, with a pore size of 0.45 micron was recommended by Goldberg *et al.* (1952) and will probably remove all "particulate" matter of any consequence from sea water. A membrane filter of similar properties has been employed by numerous workers (cf. Krey, 1939; Ballantine, 1953; Atkins and Jenkins, 1953; Jenkins, 1956; Atkins, Jenkins and Warren, 1954; amongst others) and often collects appreciably more plant material than does a fine filter paper (Riley, 1941a, b) , perhaps nearly twice as much if there are many ultraplankton (Harvey, 1950). Creitz and Richards (1955) have recommended aerosol filters (Millipore Corp. AA) which have a mean pore size that is somewhat larger than that of an H.A. filter, about 1 micron. With these filters the flow rate is greater, they clog less readily and have advantageous physical and chemical properties. The ultraplankter *Nannochloris*, with a 1 to 2.5-micron diameter cell, was quantitatively retained. There would appear to be a real danger that small soft-bodied ultraplankters can be imploded on the pores of a membrane filter and their protoplasm be sucked through. There is direct evidence for this with an AA Millipore (Lasker and Holmes, 1957) but less trouble is found with an H.A. filter.

Because all membrane filters block by a positive covering action, as each particle closes one or more holes in what resembles a smooth perforated sheet, they will retain approximately the same number of particles before the filtration rate is drastically reduced, irrespective of the initial concentration of the particles in a sample of water. Thus, if some discretion is used, the number of phytoplankton cells in a water sample need only limit the volume of water chosen for filtration and not the filtration time, which can be kept down to a relatively few minutes. Nothing is to be gained by overloading this type of filter and prolonged filtration times may even cause serious loss of material (Lasker and Holmes, 1957).

The addition of gelatinous "collecting agents" (ref. Atkins and Parke, 1951; Ballantine, 1953; Miyake *et al.*, 1954) enables filter papers to be used for the retention of even the smallest phytoplankters but offers no advantages if membrane filters are available. The latter may become seriously blocked if any such precipitates are present.

The "adsorptive filters" used by Fox *et al.* (1952), which consist of a column of adsorbant some 5 to 10 mm deep supported by paper or a glass sinter, will retain all phytoplankton. The absorbant can be made from many materials but a mixture of magnesium oxide with Hyflo-supercel appears to be one of the most satisfactory. This method of collection, however, was designed to be used for certain chemical analyses and is not very suited to the subsequent identification and analyses of phytoplankters. Much soluble organic matter will contaminate the particulate material.

3. SEPARATION OF PHYTOPLANKTON FROM ZOOPLANKTON AND DETRITUS

This problem is largely unsolved and is, perhaps, insoluble, if rapid routine methods of analysis are required that do not involve microscopy.

If one determines the total particulate organic matter by some suitable means (see later) and then the plant pigment content of this organic material, the phytoplankton can be estimated from the pigment values (Section I) and the zooplankton and animal detritus by difference (ref. Riley, 1939a; Krey, 1952; Gillbrich, 1952; Banse, 1956). The differentiation between living phytoplankton and phytoplankton detritus is at present only possible by microscopy, thus deciding what fraction of the total pigment is associated with whole cells (Gillbrich, 1952). It remains to be seen whether or not phytoplankton detritus can be estimated from the content of phaephytin *a* or phaeophorbide *a*. The analytical procedures may well be too complicated for routine application and it does not follow that the chlorophyll *a* content of detritus is necessarily all decomposed (ref. Vallentyne, 1957) or that, if decomposed, it stays for long as a phaeo pigment (Skopintsev and Bruck, 1940).

The crustacean components of the zooplankton (Calanus, euphausiids, etc.) could, ideally, be measured by the astacin-type pigments present in the plankton (Richards and Thompson, 1952). This analysis was made by Currie (1957) who reported very little pigment in the coastal waters of the northeast Atlantic in late summer.

If the primary product is measured directly by its combined carbon content, which would appear to be the most logical method (see later), any zooplankton present will be reported as plant material. The only method of separation at present practicable is based on size distribution and relies on the separation of zooplankton by retaining it on a suitably sized bolting silk mesh. Clearly this method can never give a clean separation and there will always be an overlap between the larger diatoms and filamentous algae and the smaller zooplankters, mainly larval stages and eggs.

However, if the separation is attempted using comparatively small volumes of water (a few liters maximum) filtered through unused sections of silk without suction (to minimize clogging) a separation of some efficiency could be hoped for using a No. 5 silk (0.28 mm) or even a No. 10 silk (0.16 mm). Exactly what fraction of the phytoplankton is lost by being held in these meshes and how much zooplankton and detritus passes through with the bulk of the plant cells will have to be assessed by experiment. In shallow inshore waters, in summer, detritus can present a serious problem (Riley, 1938a; Graham, 1943; Riley *et al.*, 1956). For much of the year, however, the separation might well be quite efficient (G. A. Riley, private communication) especially if nylon net is used and this is rinsed by a jet of water. Quantitative experimentation is urgently needed to test this point.

It is clear that the filter used for the final retention of phytoplankton must not be too efficient, or large amounts of colloidal organic matter will be retained (see Fox *et al.*, 1952, 1953).

No report has appeared of the use of surface properties to separate plant from animal plankton in the sense of the "flotation" techniques used commercially to separate minerals from the surrounding "gangue" rock, often of a similar particle size and density. Some form of sorting by making use of the density differences between various organisms merits a trial (cf. Anderson, 1959).

B. ESTIMATION BY COUNTING AND THE MEASUREMENT OF WEIGHT OR VOLUME

Although we are not concerned, primarily, with the classical procedures for counting and identifying phytoplankters, such procedures will always form a basis for judging other techniques and they merit some mention in the present review. The various methods, especially those concerned with nannoplankton, have been discussed by Ballantine (1953) and, more recently, in a brief review by Braarud (1957); see also the review by Lund and Talling (1957). In all this work it cannot be overstressed that the number of cells per unit sample volume (so often the only data reported) is an exceedingly poor measure of phytoplankton crop unless an elaborate weighting procedure is employed (ref. Harvey *et al.*, 1935). Even in pure cultures of the same species, the amount of material in an individual plant cell can vary by a factor of 50 or more, especially with the Bacillariophyceae. Both growth rate and food potential are functions of the total cell weight rather than cell numbers (ref. e.g. Myers, 1946b; Ketchum, Lillick and Redfield, 1949; Cupp, 1943; Gross, 1937).

Direct microscopical counting and identification are now often carried out using the "Utermöhl" technique (Utermöhl, 1931) of inspection through an inverted microscope, scanning the optically flat bottom of a glass cylinder in which the plankton are allowed to settle (ref. also H. Nielsen, 1950; Gillbrich, 1952; Holmes and Widrig, 1956; Lund *et al.*, 1958). The counting may also be carried out using the equipment employed for blood or protozoan work, such as a hemocytometer or Sedgwick–Rafter cell (Chu, 1942; Gilbert, 1942; Winokur, 1948a; Cole and Jones, 1949; Lund, 1951; Ballantine, 1953; Palmer and Maloney, 1954; Cushing, 1955; Riley *et al.*, 1956; Riley, 1957; Kutkühn, 1958). Goldberg *et al.*, (1952) have suggested that phytoplankton can be counted and examined directly on the membrane filters used for their final concentration (see also Ballantine, 1953). The organisms are killed and preserved by acetic acid-potassium iodide solution, stained with Fast Green and examined microscopically after impregnating filters with cedar oil. Holmes and Reid (1959) recommend beechwood-creosote for clearing filters. This method has the disadvantage that the organisms cannot be turned over for better inspection and some of the unarmoured flagellates and other soft-bodied nanno- and ultraplankton may not retain their shape when the filters are sucked dry. Ketchum and Redfield (1949) counted and measured the sizes of cells in pure culture by an image projection technique. Lund (1959) has recently described a simple cell for use in nannoplankton counting.

Methods for concentrating the plankton immediately prior to counting are generally based on gravity, allowing the cells in a preserved sample to settle and then syphoning or decanting off the supernatant liquid (Utermöhl, 1931; Chu, 1942; Lund, 1951; Holmes and Widrig, 1956; Riley *et al.*, 1956; Riley, 1957; Bogorov, as quoted by Laevastu, 1957) but a preliminary concentration may be made using a small-scale centrifuge and re-dispersing the cells with a little detergent to minimize "clumping" (Ballantine, 1953; Kutkühn, 1958). A membrane filter can also be used, for nanno- and ultraplankton, if the filter is not sucked completely dry and the organisms are redispersed at once in a measured small volume of water, using a soft hair brush to dislodge the plants from the membrane surface (Cole and Jones, 1949). Atkins and Park (1951) report that organisms are easily visible and are still viable when collected by a small amount of aluminium hydroxide "floc", precipitated at a pH around 7.

The statistics of direct counting methods have been dealt with by many authors (ref. e.g. Ultermöhl, 1931; Cole and Jones, 1949; Holmes and Widrig, 1956; Riley *et al.*, 1956; Kutkühn, 1958; Lund *et al.*, 1958). Concentrations greater than 100 cells/mm^3 should always be used. At least three mounts are needed from any sample with as many fields as possible counted in each mount, ideally as many as 10 fields or even the entire mount. The labour involved, especially if the size and species of each cell is required, can be truly prodigious when many samples are inspected (ref. e.g. Harvey *et al.*, 1935; Riley, 1941b; Corlett, 1953; Holmes, 1956; and McQuate, 1956; amongst others cited).

Apart from lessening the tedium of arithmetic by the use of digital computers it is difficult to see how any technicological "break-through" in this field can lessen the labour greatly, other than by some form of electronic counting method. Such methods are now being developed for use with biological problems, mainly for counting bacteria colonies, bacteria or blood cells or spermatozoa (ref. e.g. Young and Roberts, 1951; Bosselaar and Spronk, 1952; Loeser and Berkley, 1954; Mansberg, 1957) but none as yet can differentiate between different types of cells. All procedures demand extensive electronic instrumentation and no commercially available equipment is of direct applicability. The measurement of light attenuation (or scatter) is, of course, directly applicable as an arbitrary estimate of phytoplankton populations, in an analogous manner to its use with bacteria cultures (ref. e.g. Noddack and Eichhoff, 1939; Chu, 1942; Gross and Koczy, 1946; Diller and Kersten, 1954; Spencer, 1954; Kain and Fogg, 1958). Harvey (1957a) has even suggested that the turbidity of ***Phaeodactylum tricornutum*** suspensions can be employed for the bioassay of nutrients. However, natural populations are generally too sparse to be determined directly and the extinction of light is so complex a function of cell mass, shape, pigmentation, etc., that the method can only be used for measurements such as the relative growth rates of dense cultures.

If it were possible to multiply the number of all phytoplankters simultaneously and at the same rate in a mixed population by culturing, such a technique could prove helpful in the rapid identification and estimation of crop in sparsely populated waters. Such a method has found favour as a technique for the identification of species, in particular the nanno- and ultraplankton (cf. Atkins and Jenkins, 1953; Jenkins, 1956) but was considered by Ballantine (1953) to be of little value as a quantitative tool. Knight-Jones (1950), however, reported some success with a serial culturing technique (similar to that used by bacteriologists) using "Erdschreiber" and sterile sea water.

A discussion of the numerical factors that can be used to translate algal weights or the wet weights of phytoplankton into combined carbon values was presented in Section I.B. Krey (1950) actually weighed plankton directly after the filtration of 1 to 5 liters of sample through a previously dried and tared filter paper. The error was found to be almost constant at ±0.24 mg, irrespective of the plankton weight. This error could be kept to ±10% or less of the total weight if the sample volume were made sufficiently large. Very careful working was necessary, however, as the filter itself picked up water, and the technique of semi-micro weighing employed required considerable experience. A direct weighing of the plankton and detritus from 3 liters of sample is reported by Hanaoka (1957).

C. ESTIMATION FROM CARBON CONTENT

The most satisfactory method for measuring the standing crop of phytoplankton, once this is separated from detritus and animal matter, is to oxidize the plant material back to carbon dioxide, from whence it originated as a result of photosynthetic reduction. Of all the gross measures of the living tissue in the plankton this is probably the most realistic and bears the closest relation to the amount of purely organic material in the plants (in contrast with silicic acid, calcium carbonate, etc.).

The outstanding problem is one of collection, as it is perhaps unwise to use membrane filters, which have a cellulose base, for fear of contaminating the plankton with carbonaceous matter. However, this fear could be unfounded as, generally, phytoplankton can be quantitatively washed off the surface of a membrane filter, provided that it has not been allowed to suck dry, and is thus readily concentrated for most types of analysis. If a few milligrams of magnesium carbonate are first filtered onto the membrane the crop is even more easily removed (Parsons, 1959). As sintered glass or metal filters of *small* pore size but *rapid* filtration rate are not readily available, the only other obvious solution to the problem is to add the smallest practicable amount of an inorganic "collecting agent" which will entrain the plankton in a precipitate that is separated by decantation followed by centrifugation or filtration onto an inorganic-base filter. Several "collecting agents" have been found to be satisfactory for phytoplankton work, namely a preformed slurry of aluminium hydroxide (Miyake *et al.*, 1954), a floc of the hydroxide formed *in situ* by the addition of potassium alum and a little sodium bicarbonate (Atkins and Parke, 1951) and the hydroxides and carbonates that are precipitated when sodium carbonate (Laevastu, 1957) or sodium hydroxide (present author, unpublished work) is added to sea water. In the latter example a voluminous precipitate with good collection properties is formed before the pH much exceeds 9.5 and plant tissue appears to remain intact. The hydroxides and carbonates can be collected on smooth hardened papers but serious filtration difficulties may be encountered if any suction is applied and there is the ever-present danger of contamination with cellulose. Von Brand (1935) precipitated hydroxides with potassium hydroxide and centrifuged the "collected" plankton. The precipitate was dissolved in acid and reprecipitated with a little alkali to reduce its volume and to lessen the amount of absorbed *soluble* organic material.

When these precipitates, or the plankton alone after concentration by other means, are dried and burnt in a furnace the carbon content can be estimated directly by using classical micro methods for measuring the carbon dioxide that is formed (ref. von Brand, 1935; Miyake *et al.*, 1954). Such techniques are time consuming, however, and necessitate somewhat elaborate equipment and a fair degree of specialized analytical training. It is better to resort to methods of wet combustion, where the organic material is 'burnt' by means of a liquid oxidant at temperatures that do not much exceed 100°C.

Many of the oxidants used are based on work by Van Slyke *et al.* (1940), who developed an oxidant consisting of chromic oxide and iodic acid in a base of phosphoric and sulphuric acids (see also Forsblad, 1955). The iodic acid was absent in the mixture used by Lindenbaum *et al.*, 1948 (also Weisburger *et al.*, 1952; Peters and Gutmann, 1953). A ceric-chromic mixture was used by Krogh and Keys (1934) and chrome-sulphuric acid alone, similar to the well-known laboratory "cleaning mixture", has been used by other workers.

Persulphate oxidations have been recommended (Battley, 1957; Chen and Lauer, 1957) which may have an advantage in that chloride is said not to interfere. With the chromic acid oxidants chloride must either be first removed by precipitation with thallium sulphate (Krogh and Keys, 1934), by heating with phosphoric acid (e.g. Forsblad, 1955), or by scrubbing chlorine from the evolved gases using, say, potassium iodide (ref. Kay, 1954a). Interference from chloride is of practical concern, as this ion will occur to an appreciable extent even in well-washed samples of marine plankton.

The quantity of organic matter oxidized can be estimated in two ways, either from the carbon dioxide formed or from the amount of oxidant consumed. The latter is attractive when a chromic acid reagent is employed, as the amount of oxidant used up is readily measured spectrophotometrically or by titration. Generally the results are not in complete agreement with those obtained by high-temperature combustion, but a conversion factor (often about 1.1-1.2) can be applied, which is fairly constant for a given type of material (Allison, 1935; Johnson, 1949; Wakel and Riley, 1957) and varies mainly according to the amount of fat in a sample.

When carbon dioxide is to be measured, the more complex oxidants are generally used and the gas is separated in a diffusion unit (Chen and Lauer, 1957) or in a distillation unit, preferably under reduced pressure (Lindenbaum *et al.*, 1948; Kay, 1954a; Forsblad, 1955; etc.). The gas may either be determined manometrically (Van Slyke *et al.*, 1940; Battley, 1957) or by collection in barium hydroxide and subsequent titration (ref. for details: Krogh and Keys, 1934; Lindenbaum *et al.*, 1948; Weisburger *et al.*, 1952; Peters and Gutmann, 1953; Kay, 1954a; Forsblad, 1955).

D. ESTIMATION FROM NITROGEN, PHOSPHORUS AND SPECIFIC ORGANIC CONSTITUENTS

Apart from carbon, many other elements could be used as a measure of phytoplankton standing crop, provided that the amounts of these elements in the plant cells were fairly constant and were accurately known. The subject has been reviewed in Section II.A. and factors for converting the combined nitrogen and phosphorus to standing crop are suggested in Section I.B.

The main difficulty in the measurement of crop by elements other than carbon arises from the variability of the composition of plant cells, a subject discussed in earlier parts of this review. The analytical problems are not particularly serious, using modern techniques, although phosphorus is probably the more convenient element with which to work mainly on account of easier "blank" control. There are several papers describing such methods (cf. Cooper, 1934b; 1938; Harvey *et al.*, 1935; von Brand, 1935; Harvey, 1953b). Details of the micro or semi-micro analytical procedures involved are adequately covered by standard texts (ref. e.g. Pregl, 1946; Conway, 1947; Kirk, 1950) and will not be further discussed.

Recently Yentsch and Vaccaro (1958) have suggested that nitrogen may be estimated indirectly from a knowledge of the carotenoid and chlorophyll *a* content of a phytoplankton crop.

If we attempt to make use of molecular constituents as a measure of the combined carbon in phytoplankton we are severely limited by the scarcity of quantitative analytical data of any general applicability. However, were such information forthcoming, there would be attractive features about using major molecular constituents for estimating crop, as certain analytical problems might well be greatly simplified. Quite an extensive amount of work has been reported by Krey and associates on the use of the biuret albumin reaction, which gives a measure of the total plant and animal protein, both alive and as detritus (see Krey, 1951, 1952, 1953; Banse, 1956; Krey with Banse and Hagmeir, 1957). Banse assumed a mean value of about 33% for the "albumin" content of dry plankton. Another method for estimating protein in algal cells by the Folin reagent is given by Hewitt (1958a). The aldose reagent, N-ethyl-carbazol has been used to give the carbohydrate equivalent of a *Prorocentrum* sp. (Zein-Eldin quoted by Collier, 1958; Zein-Eldin and May, 1958). Hewitt (1958b) describes an application of the anthrone reagent to *Chlorella* cells. Presumably the lipid content of phytoplankton could be estimated by the very sensitive tests described by Mukerjee (1956) and Moore and Walker (1956).

The use by Aleem (1955) of triphenyltetrazolium chloride as a measure of the living tissue in marine plankton is particularly significant. This compound is reduced to a coloured form by what is said to be the dehydrogenase activity of fresh plankton and the method, although open to criticism, suggests other possibilities for the estimation of living cells (rather than detritus) by enxyme activity; perhaps certain of the enzymes predominating in plants rather than in animals could be used.

The most obvious compounds for the qualitative or quantitative assay of phytoplankters are the characteristic plant pigments, especially chlorophyll *a*. A considerable amount of work has been undertaken with pigments and this will now be reviewed in some detail.

E. ESTIMATION BY PIGMENT ANALYSIS

The reader is referred to Section II.C for an account of the plant pigments known to be present in the phytoplankton and to Section I.B for what meagre quantitative information we have concerning the chlorophyll and carotenoid content of the phytoplankters.

The spectral characteristics of the various pigments, their stability, etc., were covered by these earlier sections and will not be repeated in the present account, which is concerned only with the application of pigment analysis to the evaluation of standing crop.

The use of a plant pigment (almost invariably chlorophyll) for the quantitative evaluation of the concentration of a phytoplankton crop has been criticized by many workers (e.g. Graham, 1943; Edmondson and Edmondson, 1947; Tucker, 1949; Margalef, 1954), mainly on the grounds of the variability of the chlorophyll content of the phytoplankters and the erroneous results brought about by the presence of detrital chlorophyll. These points are well taken, and yet the method continues to be the most popular technique for crop estimation; a popularity based mainly on the speed and simplicity of the analysis and the tacit assumption that an estimate correct to little better than an order of magnitude is better than no estimation at all. Pigment analysis will always have a place amongst methods or crop estimation, but it should be extended in scope to include the carotenoids, and regularly calibrated against cell carbon so as to give meaningful and specific information. A much more critical approach is called for than has been apparent to date.

From the standpoint of spectrophotometry, ethyl ether is one of the better solvents to use for pigment extraction, but there are practical difficulties associated with this solvent; only methyl alcohol (ref. Krey, 1939; Gardiner, 1943; Kalle, 1951; Gillbrich, 1952; Banse, 1956), ethyl alcohol (Kreps and Verbinskaya, 1930), and acetone appear to have found application in marine phytoplankton work. Anhydrous acetone gives very inefficient extraction but it can be used quite wet, with little control of the water content (Atkins and Parke, 1951), or diluted with 20% of water (Graham, 1943; Edmondson and Edmondson, 1947; Atkins and Jenkins, 1953; Jenkins, 1956; Steel, 1956). The use of a 90% (v/v) acetone–water mixture now finds favour (Richards, 1952; Riley *et al.*, 1956; Davis, 1957; Odum *et al.*, 1958), although there is still some doubt as to the time required for extraction. Graham (1943) extracted phytoplankton for only 30 minutes but at least 9 hours is reported to be necessary by Richards (1952) and 18 hours by Davis (1957). Gardiner (1943) reported colour in some Chlorophyceae and Myxophyceae after 14 to 20 hours and recommended the use of a mixture of methyl alcohol and benzene for these classes of algae.

The need for preventing acidity in samples and extracts (by the addition of week organic or inorganic bases) and for storage under refrigeration in the dark has already been stressed and the reader is referred to a paper by Zscheile *et al.* (1944) for much practical information on the stability of pigment extracts. Krey (1939) suggested that the stability of the pigments in phytoplankton cells could be increased by exposing the filtered cells to steam for a few moments so as to inactivate the enzyme, chlorophyllase.

Numerous instruments have been used for measuring the light absorption of pigment extracts. Simple absorptiometers are satisfactory in certain circumstances but a spectrophotometer is almost mandatory if differential pigment analysis is to be attempted by making measurements at several wavelengths. Although the Beckman DU spectrophotometer has proven itself to be a seaworthy instrument, pigment analysis need not necessarily be undertaken at sea, as filters containing the phytoplankton can be stored in a desiccator in the dark under refrigeration for several weeks. The main requirement is for a micro or semi-micro curvette, which will contain the smallest practical volume of extract in the longest practical cell. A 10-cm cell holding about 10 ml of extract will enable a measurement to be made of as little as 0.2 to 0.3 micrograms of chlorophyll *a*, corresponding approximately to 0.01 mg of phytoplankton carbon. Thus a volume not exceeding 5 liters gives sufficient sample for analysis when the crop density is at its lowest and the analysis can be conveniently carried out using a liter or less of sea water for much of the year in inshore areas. The carotenoid pigments have a somewhat greater spectral sensitivity than chlorophyll *a* but, as the carotenoids are present in phytoplankton in amounts that are less than a half of the chlorophyll *a*, sample volumes cannot be reduced.

The credit for first using a pigment extraction method for the estimation of marine phytoplankton is generally given to Kreps and Verbinskaya (1930), who applied chlorophyll analysis to the measurement of the phytoplankton in net hauls. Standardization against pure chlorophyll *a* was used by Krey (1930), Graham (1943), Edmondson and Edmondson (1947), Gillbrich (1952), Steel (1956) and Banse (1956), but several workers have employed commercial chlorophyll (e.g. Riley, 1941a, b; Atkins and Parke, 1951; Atkins and Jenkins, 1953; Jenkins, 1956; Riley *et al.*, 1956), which invariably contains chlorophyll *b* and pheopigments as impurities. Gillam *et al.*, (1939) used a mixture of pure chlorophyll *a* and *b* but there would appear to be no justification for such a procedure. The use of an impure pigment for calibration can be justily criticized (Gardiner, 1943) but much depends upon the precision of the results and their interpretation. In comparison with other possible sources of error the purity of the chlorophyll standard is probably a minor consideration.

An even less accurate standardization procedure was initiated by Harvey (1934a) who resorted to the visual matching of extracts of net phytoplankton in 80% acetone with a standard of nickel sulphate and potassium chromate in water. Nickel chromate can also be used (Atkins and Parke, 1951). This method led to the reporting of mixed chlorophyll and carotenoid pigments in terms of HPP units (see Section I.A). Graham (1943) has criticized the arbitrariness of such a method. Although it originally had something to recommend it for field work, where the absence of a colorimeter or stable chlorophyll standards might otherwise have prevented any measurements being undertaken, nevertheless the procedure introduces yet another uncertainty in the chain of calculations leading to final standing crop values (Section II.B). In retrospect it is to be regretted that so many field observations are now available only in the form of Harvey Plant Pigment Units (see for example, Harvey, 1934a, b; Harvey *et al.*, 1935; Riley, 1938a; Riley, 1939a, b; Riley, 1941b; Riley and Gorgy, 1948; Tucker, 1949).

Riley (1938b; 1939a) effected a separation of carotenoids from chlorophyll by an extraction procedure, mainly in an endeavour to render natural pigment extracts more readily matched with chlorophyll standards. Gillam *et al.* (1939) carried out qualitative tests and chromatographic separations of plant pigments on a mixed phytoplankton–zooplankton concentrate. Krey (1939) measured the extinction of extracts at two different wavelengths in order to evaluate both chlorophyll *a* and carotenoids, separately, and this method was subsequently employed by Gillbrich (1952). It remained, however for Richards with Thompson (1952), using the quantitative spectra determined by Richards (1952), to perfect a method for the separate evaluation of some of the different pigments extracted from marine phytoplankton by 90% acetone.

In theory, it is only necessary to know extinction values at as many wavelengths as there are separate pigments present in a solution for the pigment composition of the solution to be resolved completely. However, in practice, even if a spectrophotometer with a good resolution is available and the individual spectra of all pigments are known, the method is limited to those spectra showing a moderate separation of absorption maxima, say at least 150-200 A for the normal type of band. For this reason the equations of Richards with Thompson (1952) only enable separate estimates to be made of chlorophylls *a, b* and *c,* from measurements 6650 A, 6450 A and 6300 A. If additional readings are taken at 5100 A and 4800 A, a measure of the total plant carotenoids and "astacin type" carotenoids of Crustacea (ref. Kuhn and Lederer, 1933) can be obtained. However, no satisfactory resolution of the carotenoids to carotenes and xanthophylls is possible and the precision of chlorophyll *c* measurement may be little better than ±30%. If a separate estimate of the various carotenoid pigments is desired, some supplementary separation by chromatography (e.g. Purcell, 1958) or extraction must be attempted (ref. Haagen-Smit *et al.,* 1943, for a separation of carotenes from xanthophylls by extraction). Such procedures would greatly detract from the speed and convenience of a pigment analysis but sufficiently valuable information might result from a detailed analysis of the xanthophylls (ref. Section III.B) to justify the extra labour. With the new techniques of centrifugal chromatography the time factor is no longer serious.

The Richards-Thompson method, especially as modified by Creitz and Richards (1955) for direct use with membrane filters, has been understandably popular and will probably form the basis for most future methods. Several investigations using the technique have been described in recent years (e.g. Yentsch, 1955; Marshall, 1956; Currie, 1957; King *et al.,* 1957; Holmes *et al.,* 1957; Holmes, 1957) and it would now appear to be almost universally employed at institutes in North America. Convenient nomographs for use with the method have been issued by Duxbury and Yentsch (1956). The only unsatisfactory aspect of the procedure is the evaluation of chlorophyll *c* and carotenoid pigments in arbitrary SPU values (see Section I.A) instead of in actual pigment weights. The MSP unit is doubtless close to a miligram but it is to be hoped that conversion factors will soon be forthcoming that will render the continued use of the MSPU unnecessary.

The Richards-Thompson method does not give a sufficient differentiation of pigments to enable taxonomic sorting, as envisaged by Gardiner (1943), but quite instructive information is accumulating on the distribution of chlorophyll *c*. The ratio of chlorophyll *c* to chlorophyll *a* is reported by Currie (1956) to be greater in the open ocean than at the coast and the ratios found by King, Austin and Doty (1957) for the tropical Pacific Ocean exceed values found in the northeast Atlantic. It is inferred that these ratios reflect changes in the taxonomic composition of the plant populations.

The fluorescence method developed by Kalle (1951) for estimating the chlorophyll in extracts is reputed to be very sensitive but does not appear to have achieved much popularity, probably because the necessary instrumentation is not very familiar to most workers. A direct measurement of the absorption of chlorophyll in a concentrated suspension of living cells could be attempted but standardization is difficult and several errors of measurement can arise in the spectrophotometry of suspensions (ref. e.g. Latimer, 1957). Yentsch (1957) has described an ingenious method for the measurement of the chlorophyll in the phytoplankton filtered on to a millipore membrane filter. The filter is oiled with cedar oil and pressed between glass slides in a special holder fitted into the spectrophotometer. The chlorophyll, *in situ* in the plant cells, is measured at 6700 A and the scatter and absorption brought about by the filter are then allowed for by making a further measurement at 7500 A. The method is calibrated against the Richards-Thompson procedure and is said to have practically the same sensitivity, but some precision is sacrificed in exchange for increased speed and convenience.

Finally a plea might be made for the greater use of caroteniod analyses in crop estimation. The extra labour involved, when using the Richards-Thompson technique, is relatively small and there is some indication that these pigments may have a more constant ratio to the combined carbon than does chlorophyll *a* (Yentsch and Vaccaro, 1958; see also Sections II.C and IV.G).

In view of the well-established application of aerial photography to marine work for recording the movement of turbid river outflows, or locating patches of coloured 'tide' organisms, it would appear worthwhile to attempt its use for studying the quantity and distribution of all phytoplankton organisms, especially in productive coastal areas. The advantages of such a technique, if it could even be made semi-quantitative, are obvious. Probably the only way in which phytoplankton patches might be delineated and the concentration of plant matter assessed would be by narrow wave-band photography at several wavelengths, both near the chlorophyll and carotenoid peaks and at a wavelength of minimum absorption. The writer has seen no reference to such work having been attempted but the aerial photography of Moore (1947) is relevant.

F. SOME RESULTS OF STANDING CROP DETERMINATIONS

It is not the object of this review to attempt a world coverage of the many results obtained from standing crop measurements. The values quoted in the literature vary widely and are of little interest, in detail, except to workers in a particular sea area.

In subarctic regions a general picture emerges of several maxima or "blooms" of phytoplankton throughout the year, the greatest in early spring, in March or April, followed by secondary maxima at intervals of 1 or 2 months. An autumn bloom in October, or even November, can usually be detected and there may be a period in August where the standing crop is very low, a tenth or less of the annual maximum. This minimum in August is presumably the result of gross nutrient depletion above a well-established thermocline. A winter minimum between December and February can be attributed to a shortage of radiant energy. This minimum, however, is often less pronounced than the midsummer depletion of crop. The result of zooplankton grazing is superimposed upon the patterns given by purely physical and nutrient factors, to make a complex picture which varies from one sea area to another and, indeed from one year to another. During one 12-month period both the times and magnitudes of blooms may vary to a marked extent from those of the previous year. Subsidiary maxima which are well marked in one year, may not appear at all during the following 12 months.

In the open ocean, particularly in tropical seas, the crop values are much less variable with time, although considerable changes undoubtedly occur. The plant concentration is smaller in the tropics than in arctic waters but the total crop beneath unit area may well be as great (Riley, 1939b; Riley and Gorgy, 1948; Riley, 1957). Certain aspects of the standing crop pattern and its prediction will be dealt with in Section V.

The distribution of phytoplankton with depth is even less predictable and is often difficult to assess because of the presence of detritus. There is generally a maximum crop near the surface with a secondary maximum near to the bottom of the euphotic zone. A third concentration may be found at the thermocline when this is below the euphotic zone but much of the material there is presumably detrital or consists of senescent and dying plant cells (ref. e.g. Gessner, 1949; Riley *et al.*, 1949; Gillbrich, 1952; Riley, 1957; Holmes *et al.*, 1957). The pattern is very dependent upon local conditions and upon the time of year (Braarud *et al.*, 1958).

Reference should be made to the literature for details of the species of phytoplankters present in the crops of different sea areas. In the Sub-arctic the spring bloom consists mainly of large diatoms which are succeeded by smaller species of the Bacillariophyceae, Xanthophyceae and Chrysophyceae with more exacting nutrient requirements. As the year progresses members of the Dinophyceae and Chlorophyceae become more abundant, localized blooms of the former being characteristic of the summer and autumn months.

The crop values collected in Table VII have been selected from only a small number of published papers and often only a few values are given from any one paper. Some of the earlier values, relying on vertical net hauls, can only be a fraction of the true crop, perhaps little more than 10%. It is unfortunate that, even in recent years, the results of several painstaking investigations cannot be translated into crop weight, as only cell numbers are reported (e.g. Corlett, 1953; Ragotzkie and Pomeroy, 1957; Smayda, 1957).

The present author has made free use of the factors suggested in Section I.B to convert the published experimental data into crop weights. The results may often differ from those calculated by the original authors but they are only intended to illustrate magnitudes and no great accuracy is necessary for this purpose.

TABLE VII. Selected examples of crop measurements.

Location	Approximate depth of maximum crop	Depth of sample	Time of year	Crop		Method	Reference	Remarks
				mg C/m^3	mg C/m^2			
Near Plymouth, England		Average for euphotic zone	March (max.)	50*	....	HPP units	Harvey, 1934b	*Net hauls; figures are low.
			August (min.)	1*	....			
			November (max.)	30*	....			
			January (min.)	5*	....			
Near Plymouth, England		Average for euphotic zone	March (max.)	25*	....	HPP units	Harvey, Cooper *et al.*, 1935	*Net hauls; figures are low. Details of species given.
			September (max.)	20*	....			
			December (min.)	1*	....			
Near Plymouth, England			June (max.)	....	4,000	HPP units	Harvey, 1950	......
			July (min.)	....	600			
			December (min.)	....	700			
20 miles off Plymouth, England		Surface	January (min.)	10	....	Chlorophyll	Atkins and Parke, 1951	......
			July (max.)	150	....			
			August (min.)	20	....			
			November (max.)	40	....			
20 miles off Plymouth, England	0 and 20 m	Surface	December (min.)	100	....	Chlorophyll	Atkins and Jenkins, 1953	These results appear to be very high. Details of species given.
		50 m	December (min.)	100	....			
			December (min.)	....	6,000			
	"	Surface	Mar-Apr (max.)	1000	....			
		50 m	Mar-Apr (max.)	50	....			
			Mar-Apr (max.)	....	45,000			
20 miles off Plymouth, England	Surface in Sept. Generally a slight max. at about 20 m	Surface	April (max.)	1000	75,000	Chlorophyll	Jenkins, 1956	These results appear to be very high. Details of species given. Jan. min. is for following year.
		Surface	August (min.)	150	15,000			
		50 m	March (max.)	1500	....			
		50 m	June (min.)	100	....			
		50 m	January (min.)	100	6,000			
Southeast North Sea	......	......	March	....	600–700	Chlorophyll	Banse, 1956	Considered to be minimum for year.
Kiel Bay, North Germany	Uniform 10—15 m	Average for euphotic zone	January (min.)	80	....	Chlorophyll	Gillbrich, 1952	......
			April (max.	250	....			

TABLE VII. (Continued)

Location	Approximate depth of maximum crop	Depth of sample	Time of year	Crop		Method	Reference	Remarks
				mg C/m^3	mg C/m^2			
North Sea	......	Average for euphotic zone	Late April Late May	55 25	....	Number and size of plant cells	Cushing, 1957b	......
North Sea	......	Average for euphotic zone	May (max.)	5*	....	Chlorophyll	Gillam *et al.*, 1939	*Net hauls. Figure is low. Details of species given.
North Sea	10—20 m	10 m	May (max.)	100	3,000	Chlorophyll	Steel, 1956	Some data given for repeat sampling.
Long Island Sound, east U.S.A.	Fairly uniform	Average for euphotic zone	March (max.) 1952 March (max.) 1953 Winter values	300 600 100	....	Chlorophyll	Riley *et al.*, 1956	Contribution by S.M. Conover.
Long Island Sound, east U.S.A.	......	1 m	Winter min. Spring max.	150 3100	....	Chlorophyll	Riley, 1941a	......
Off New England Coast, U.S.A.	......	Average for euphotic zone	March	100	....	Chlorophyll	Quoted by Ketchum, 1957	......
Off New England Coast, U.S.A.	......	Surface	August	40–200	....	HPP units	Riley and Gorgy, 1948	......
Northwest Atlantic	Top 25 to 50 m	Surface	Summer	40	1,500	HPP units	Riley, 1939b	......
Northwest Atlantic	Top 30 m 30 m Uniform to 50 m	Average for euphotic zone	April (max.) May (min.)	800 300 40	....	HPP units	Riley, 1941b	Author's own factor taken. Details of species given.
North Central Sargasso Sea Atlantic Ocean	Generally 50—100 m	Average for euphotic zone	June (sharp max.) Approx. mean value for most of year	150 25	....	Algal volume	Riley, 1957	Details of species given.

TABLE VII. (Continued)

Location	Approximate depth of maximum crop	Depth of sample	Time of year	Crop		Method	Reference	Remarks
				mg C/m³	mg C/m²			
Sargasso Sea Atlantic Ocean	Around 100 m	Surface ?	Highest value on cruise.	*ca.* 3	1,000	HPP units	Riley and Gorgy, 1948	......
			Lowest value on cruise	*ca.* 1	50			
Caribbean Area	Fairly uniform in top 200 m	Surface	Summer	15	5,000	HPP units	Riley, 1939b	......
East Gulf of Mexico	In top 15 m and at 150 m (thermocline)	Average for euphotic zone	July	15	....	HPP units	Riley, 1938a	Taken from *text*. Presumably a misprint in the figure.
Inshore waters off Florida, U.S.A.	......	Surface	Summer average	400	....	Chlorophyll	Marshall, 1956	......
			September (max.)	650	....			
California Coast, U.S.A. Inshore	......	Surface	August	50	....	Chlorophyll	Graham, 1943	Detailed account of species.
Equatorial Pacific. Productive area near coast of Ecuador	Surface	......	November	35	....	Chlorophyll	Holmes *et al.*, 1957. See also Holmes, 1957	......
Much of open ocean Pacific in equatorial belt north of equator	Values at 40 to 70 m generally about twice those at 0 m	Surface	*Very approx. value for autumn*	10	....	Chlorophyll	Holmes *et al.*, 1957. See also Holmes, 1957	Large number of data reported.
Equatorial Pacific east of 160° W	......	Near surface	November	Min. *ca.* 5 Av. *ca.* 20 Max. *ca.* 150	....	Chlorophyll	King *et al.*, 1957	Large number of data reported.
Northeast Pacific. Productive area near end of Aleutian chain	......	Surface	November	200	....	Chlorophyll	Holmes, 1957	......

TABLE VII. (Continued)

Location	Approximate depth of maximum crop	Depth of sample	Time of year	Crop		Method	Reference	Remarks
				mg C/m³	mg C/m²			
Northeast Pacific Oceanic area 55° N, 155° W	......	10 m	August–September	20	....	Chlorophyll	Yentsch, 1955	......
Northeast Pacific off Alaska Panhandle 55° N, 135° W	......	10 m	August–September	60	....	Chlorophyll	Yentsch, 1955	......
Northeast Pacific off Kodiak Island 57° N, 153° W	......	10 m	August–September	50	....	Chlorophyll	Yentsch, 1955	......
Off coast of Japan	......	Surface	August	1000–2000	....	Direct combustion	Miyake *et al.*, 1954	Values almost certainly high due to inclusion of zooplankton and detritus.
Off coast of Japan	......	?	Annual range	15–75	....	Chlorophyll	Hanaoka, 1957	Chorophyll data not reported. Author's own conversion used.
Indian Ocean Section of Antarctic waters, between pack ice and Antarctica	0—10 m and 25 to 50 m	0—10 m	Summer	Very approximately 200*	....	Wet weight	Brodsky and Vinogradov, 1957	*Net hauls. Figure is low. Very uncertain conversion to carbon.

SECTION IV. MEASUREMENT OF THE RATE OF PHOTOSYNTHESIS

In this section we will restrict the discussion of productivity measurements mainly to the procedures used. A consideration of certain other aspects of marine productivity and its prediction in nature will be found in the concluding part of this review (Section V.C). In particular, we will postpone until then any mention of the complex relationships that exist between the results obtained in small scale experiments on board ship and the actual *in situ* production of phytoplankton in the euphotic zone.

The correct interpretation of much of the experimental data in the field of photosynthesis measurement depends on knowing the correct relation between photosynthesis and the simultaneous respiration that takes place in a plant cell. This relationship will therefore be reviewed at some length before discussing the main topics in the present section. The subject is so complex, and the object of so many investigations, that it will be necessary to limit the present review to a few of the more recent and relevant aspects that are possibly applicable to marine phytoplankton.

A. PHOTOSYNTHESIS AND RESPIRATION

Any account of the detailed mechanism of photosynthesis, especially the nature of the biochemical cycles involved, is out of place in the present review. In addition to the comprehensive text by Rabinowitch (1945, 1951, 1956) there have been reviews in the last decade, appearing at the rate of several a year, which cover the subject as thoroughly as could be desired. Only a limited number of these reviews, however, can be classed as brief and introductory. Very readable accounts are given by Calvin (1953), Gaffron (1954), Wittingham (1955a, 1955b), Aronoff (1957), Bassham and Calvin (1957), Arnon *et al.* (1958), Arnon (1959).

1. Oxygen and Carbon Dioxide Requirements

The inhibition of photosynthesis brought about by oxygen deficiency is not appreciable unless algal cells are subjected to several hours of anaerobic conditions (ref. Rabinowitch, 1956) and such inhibition is presumably out of the question in any normal marine environment. Photosynthesis is also inhibited by the presence of excess oxygen. This inhibition in *Chlorella* is reversible and depends upon the concentration of carbon dioxide also present, being more marked at lower carbon dioxide concentrations (Tamiya and Hujishige, 1949). If the data for *Chlorella* are at all representative of algae in general some inhibition might be expected at the surface of the euphotic zone, in waters supersaturated with dissolved oxygen, but the effect could only be slight (less than 5-10%) and is presumably of no practical significance.

The inhibition brought about by excess carbon dioxide does not take place with algae unless the concentration of the gas locally exceeds about 10^{-3} M; clearly this is outside the range to be expected in any marine environment. The question of inhibited photosynthesis at low carbon dioxide concentrations is more complex and is tied in with considerations of the forms of carbon dioxide that are available to aquatic plants. There has never been any serious suggestion that the divalent carbonate ion, CO_3^{2-} could be a direct source of carbon dioxide. There has been much controversy, however, as to whether the bicarbonate ion, HCO_3^{1-}, can penetrate the cell membrane of a plant and act as a direct source of carbon or whether only dissolved carbon dioxide gas is used and the ion HCO_3^{1-} simply acts as a labile source CO_2 by *extracellular* dissociation.

The problem has been reviewed by Rabinowitch (1956) and the reader is referred thereto for details. Information has been obtained mainly by studying the rate of photosynthesis in solutions containing widely differing concentrations of HCO_3^{1-} and dissolved CO_2 but the main difficulties arise in the interpretation of the data so obtained. From the work of Osterland, Wittingham, Gaffron, Steeman-Nielsen and others a clearer picture has recently emerged and it would seem that both CO_2 and HCO_3^{1-} can participate. As usual with these problems of algal physiology, much of the work has been undertaken using freshwater Chlorophyceae and one is left in considerable doubt as to what generalizations are possible to cover marine conditions.

Dissolved carbon dioxide is undoubtedly the favoured compound for carbon transfer across the cell membrane but some aquatic plants appear to have acquired the ability to utilize bicarbonate, especially those whose natural habitat is in an alkaline environment. The adaptation most probably arises from changes in membrane permeability, rather than any basic change in photosynthetic mechanism, and the HCO_3^{1-} ion, once inside the chloroplast, simply acts as a source of the primary CO_2-complex, *via* a chain of rapidly adjusting equilibria.

Nielsen has suggested that the deciding factor may be the dimension of the liquid diffusion path into the cell. Where this is only a micron or less, carbon dioxide is probably transported at an adequate rate, whatever its extra cellular concentration. With longer path lengths a plant cell may be able to adapt from one transport mechanism to the other, according to the relative abundance of CO_2 and HCO_3^{1-}. Bicarbonate adaptation has been reported when the dissolved carbon dioxide concentration was below about 5×10^{-5} M. In sea water, where the carbon dioxide concentration is around 2×10^{-5} M in the presence of a hundred times this concentration of bicarbonate, either a CO_2 of HCO_3^{1-} mechanism might predominate and much would depend on the species of algae. However it might be expected that most marine plants would by now have adapted to a bicarbonate mechanism and some evidence for this is suggested by the experiments of Park (1957).

No lower limit of carbon dioxide concentration, below which the rate of photosynthesis was retarded, could be found by Weigl *et al.* (1951) but such a limit must, in theory, exist, depending upon the transport conditions in any experiment, and is probably around 10^{-5} to 10^{-4} M for most algae (Rabinowitch, 1951, 1956). The large carbonate reserve of the marine environment would presumably mask any effect in the sea, where the carbon dioxide concentration is never considered to be limiting. Whether or not "carbonate fertilization" might enable some marine algae to grow more rapidly than is normal does not seem to have been checked. This would be most unlikely in shaken cultures.

There will be a "carbon dioxide compensation concentration", depending upon the light intensity, at which the rate of CO_2 utilization just equals the rate of respiration. This concentration has been reported to be near to 10^{-6} M at high light intensity and thus would be encountered but rarely in the sea. The concept is, of course, unimportant if bicarbonate utilization occurs.

The possibility that some of the carbon dioxide in sea water may be present in a readily available carbamino-complex cannot be overlooked (Neuberg *et al.*, 1957). Such complexes carry some of the carbonate in mammalian blood (ref. e.g. Stadie and O'Brien, 1936) and a glutamic acid complex appears to be important for photosynthesis by *Chlorella* (Warburg, 1958). There is recent evidence of carbonate in the carbamino form being used by the marine phytoplankters of inshore waters rich in organic matter (Smith, 1958).

2. PHOTOSYNTHETIC AND RESPIRATORY QUOTIENTS

The instantaneous values of photosynthetic and respiratory quotients (ref. Section I.A) may vary enormously, due to short-term delays in overall plant metabolism, "CO_2 bursts", etc. These variations, which persist over periods of a few minutes or less, are of the utmost importance when studying the kinetics of photosynthesis and photosynthetic quantum efficiency but in the present review we are mainly concerned with the mean long-term quotients, values which are to be used to correct experimental data measured over a period of several hours.

Under these conditions the PQ must, theoretically, equal unity for hexose synthesis or about 1.4 for "fat" synthesis (glyceryl tripalmitate). If a protein (albumin) is formed with ammonia as the source of nitrogen the PQ should equal about 1.05 but will be as high as 1.6 if nitrate is the source.

The form of combined nitrogen used in photosynthesis and the fat content of the final cells are thus the main factors determining the PQ (ref. Spoehr and Milner, 1949, and the review by Syrett, 1954). If ammonia is present as well as nitrate, the former is generally used first and nitrate is only assimilated when the ammonia is exhausted (Cramer and Myers, 1948). At moderate light intensities a PQ of 1.5 is found with nitrate, compared with 1.05 if ammonia alone is present, the enhanced PQ value, $\frac{+\Delta O_2}{-\Delta CO_2}$, being brought about by a decrease in the rate of carbon dioxide consumption rather than by an increase in the rate of oxygen evolution. (This would be expected if both NO_3^{1-} and CO_2 were competing for reduction by a limited amount of radiant energy (see also Syrett, 1956b).

At high illumination and with an excess of carbon dioxide present Van Niel *et al.* (1953) report that the rate of carbon dioxide assimilation is not reduced in the presence of nitrate (cf. the low light intensity effect just mentioned) but rather the oxygen evolution rate increases. In these circumstances carbon dioxide is *not* formed by the oxidation of cell material by nitrate (cf. Warburg and Negelein, 1920) but nitrate presumably acts as an alternative acceptor for photosynthetically produced hydrogen atoms.

The experimental results (for *Chlorella*) agreed excellently with the PQ values predicted from chemical analysis of the plant cells (Cramer and Myers, 1948).

Myers and Cramer (1948) have pointed out that the normal condition of a cell is probably that found when a culture is growing in a well-balanced nutrient medium at low light intensity. If the cell is nutrient starved, the PQ will be low (about 1.1), especially with a high light intensity, whilst the cell builds up its complement of carbohydrates by an "overflow" mechanism. If the light intensity is decreased after a prolonged period of illumination a very high PQ can result (up to 2.5) because carbohydrate glutted cells re-synthesize protein, partially at the expense of existing hexose (Myers and Cramer, 1948).

Most of the PQ values quoted in the literature for Chlorophyceae and Bacillariophyceae are very low, being around 1.05 (see Barker, 1935; Fogg, 1953; and Ryther, 1956a, for lists), which is remarkable, considering the average chemical composition of the algae (Tables I and II). As Ryther (1956a) has pointed out, however, many of these results were obtained from experiments conducted at a relatively high light intensity. Under most marine conditions a PQ of 1.25 or even 1.33 has been suggested (Nielsen, 1952a; Ryther, 1956a). In practice, of course, the respiration of bacteria and zooplankton in natural populations will effectively reduce the measured PQ and this probably explains the lower values found by McQuate (1956) and Sargent and Hindman (1943). An approximate working value of 1.2 was suggested in Section I of the present review as being preferable to the practice of assuming unity for this quotient.

Factors affecting the respiratory quotient are similar to those affecting the photosynthetic quotient. Carbohydrate, rather than lipid material, appears to form the substrate for most respiratory processes, as witnessed by the fact that the RQ stays near to unity even in starved cells (Myers, 1951; Fogg, 1953). In *Chlorella* the RQ, which is near to 1.0 with ammonia as a source of nitrogen, may reach 1.6 or greater in a nitrate-containing medium (Warburg and Negelein, 1920; Cramer and Myers, 1948; Kessler, 1953; Syrett, 1956a), the production of "extra carbon dioxide" accompanying nitrate assimilation. Very high values for the respiratory quotient (3.3) were reported by McQuate (1956) but here, as with abnormally low PQ values, it is necessary to distinguish between the true quotient and experimental values which may be in error due to the respiration of extraneous organisms.

Finally, we should bear in mind the loss of cellular matter that can occur from the excretion of soluble organic matter by living algae. Such a loss of combined carbon brings about an *effective increase* in both PQ and RQ if we use these ratios to convert oxygen changes to the net synthesis or loss of *cellular* carbon.

Algae will, of course, lose water soluble organic matter rapidly on death, even if preserved (Spoehr and Milner, 1949), and Krogh *et al.* (1930), working with *Scenedesmus* and mixed freshwater phytoplankton, found that up to 10% of the synthesized carbon compounds in living cells were lost to the surrounding water. Such values appear quite normal for the Chlorophyceae and Myxophyceae (Fogg, 1952 and 1953). The mucilaginous freshwater flagellate *Chlamydomonas*, studied by Lewin (1956), excreted up to a quarter of the organic matter it synthesized as a water-soluble polysaccharide (ref. also Aleyev, 1934; Bishop *et al.*, 1954; Fogg and Westlake, 1955; Allen, 1956; Guillard and Wangersky, 1958; amongst others). Kay (1954) has suggested that a considerable amount of the soluble organics found in the sea originate as secretions from phytoplankters (ref. e.g. Gaarder and Gran, 1927; Currie, 1957) but the relative rates of excretion and synthesis by phytoplankters in a marine environment have not been quantitatively determined. Fogg, who has long drawn attention to this problem, estimates from experiments in lakes using ^{14}C (Fogg, 1957a) that errors due to secretion may exceed 5% if photosynthetic measurements are prolonged much over 6 hours. The matter was also discussed by Rodhe (1956) when describing his studies of productivity in Swedish lakes and further evidence is given by Fogg and Boalch (1958).

By analogy with the above, an *effective decrease* of the PQ arises if heterotrophic growth occurs in marine phytoplankton. Although such growth is well established for certain Chlorophyceae and has been shown to be possible for freshwater diatoms (Lewin, 1953), there is little direct evidence for its occurrence under normal marine conditions. However, such growth might be appreciable, especially in shallow tropical areas, and errors would thus be introduced were "productivity" judged solely on the basis of photosynthesis (see Wood, 1956; Odum and Hoskins, 1958).

3. THE RESPIRATION OF ALGAE DURING ILLUMINATION

Numerous factors affect the respiration of plants, but except for temperature, which increases the respiration in an exponential manner for a limited temperature increase, not much quantitative information is available for marine phytoplankton. The past history of cells is known to be important. Respiration appears to be very low during the "lag" phase of culture growth and during much of the exponential growth stage but increases as cells age (Riley, 1943;

Kok, 1952; Ryther, 1954a). Recently liberated daughter cells of *Chlorella pyrenoidosa* respired at only about one-third of the rate found with mature cells (Sorokin and Myers, 1957).

Much of the decrease in the net/gross photosynthesis ratio that arises from nutrient deficiencies would appear to result from effects on gross photosynthesis and not respiration (Kok, 1952; Ryther, 1954a, 1956c; Ketchum, 1957). With the exception of certain obvious respiratory poisons, mentioned later in this section, it is not clear what depressive action, if any, nutrient adnormalities may have on the respiration of marine phytoplankters.

Our main concern in marine productivity measurements, however, is the extent to which respiration alters when plant cells are illuminated (see review by Wittingham, 1955b). The two process of respiration and photosynthesis were early considered to be completely independent (ref. Gaffron, 1939; Warburg *et al.*, 1949). However, respiration rates measured immediately after a period of illumination were often found to be different from those found before the illumination was first switched on.

Thus Emerson and Lewis (1943) reported a "respiration anomaly" when dark-adapted cells were illuminated for more than a few minutes. The respiration rate was found to have increased by the commencement of the next dark period, as if the rate had been slowly increasing during the period of illumination. Brackett *et al.* (1953) found that the respiration of cells during prolonged periods in the dark (many hours) fell to a very low value but increased some 5-fold if the cells were exposed to light for a few minutes before again returning them to the dark. Much of this enhancement was short lived, decaying in 10 to 30 seconds and then at a more moderate rate for the next few minutes. A small residual effect took many hours to die away. With a series of light and dark spells, each of a few minutes duration, much less enhancement occurred but it appeared as if respiratory demands exercised priority on the utilization of one or more products of photosynthesis, especially on one or more short lived intermediates (ref. also Weigl *et al.*, 1951). Verduin (1957) reported that an enhanced respiration rate (nearly double) persisted for several hours after exposing a natural population to optimal light intensities.

The exact pattern of respiration during illumination is open to doubt in all these "classical" experiments, where respiration must be measured in the absence of light, even if the dark spells are of short duration.

If isotopically labelled oxygen or carbon dioxide is used, however, the respiration can be followed *directly* whilst plant cells are exposed to light. Using an atmosphere enriched with ^{18}O, Brown (1953) was able to show that respiration was, in general, the same in the light as in the dark (ref. also Van Norman and Brown, 1952). Brief and transient "respiration anomalies" were found when the light was extinguished but these effects were mainly conditioned by the past history of the cultures *(Chlorella)*, being most marked with cells grown with a deficiency of oxygen. There was no indication of any marked enhancement.

A "photosynthetic anomaly", the converse of a "respiration anomaly", was reported by Joliot (1957), who showed that an oxygen "burst" occurred on illuminating dark-adapted cultures. The phenomenon persisted for only a few seconds, however, and probably has no significance in marine productivity measurements.

There have been numerous indications that at very low light intensities, below the compensation point, the quantum efficiency of gross photosynthesis increases, which has implications of interest in the marine environment near the base of the euphotic zone. This phenomenon, the Kok effect (Kok, 1948, 1951) shows up as an increase in the slope of the curves of respiration vs. light intensity, below the compensation point, although it is rather elusive. The *extraplorated* gross photosynthesis rate appears to be positive even at zero light. Brackett *et al.* (1953) confirmed the Kok effect only when corrections for respiration in the light were made from average dark period values. If short-term respiration anomalies were ignored gross photosynthesis was a linear function of light down to zero light intensity. If this is indeed the case, a value for respiration in the light can be obtained from an extrapolation of the curve of net photosynthesis vs. light intensity down to zero light (Nielsen, 1957a). The Kok effect has been attributed to photosynthesis taking place from a pool of partially reduced respiratory intermediates, rather than directly from carbon dioxide, and to the oxidation of some such compounds (Franck, 1949; Gaffron, 1954).

Working with $^{14}CO_2$, Weigl *et al.* (1951) found that respiration in the light was sometimes variable and depended upon the external concentration of carbon dioxide, as if *intra-cellular* CO_2 were being reused for photosynthesis before it left the organism. This effect is of the greatest importance for the correct interpretation of productivity measurements and merits a fuller discussion.

4. PARTICIPATION OF INTRA-CELLULAR MATERIAL AND THE INTERPRETATION OF RADIOCARBON PRODUCTIVITY MEASUREMENTS

As the final product of respiration, carbon dioxide, is also consumed during photosynthesis and, conversely, oxygen is both produced by photosynthesis and used up for respiration, then it seems reasonable to suppose that the carbon dioxide or oxygen formed by one mechanism may be used up by the other, without the gases ever leaving the plant cell. The participation of intra-cellular material, rather than oxygen or carbon dioxide from outside the cell, will depend upon the location of the various processes within the plant, the extra-cellular concentration of gases and the transport properties of the cell membrane. Assimilation will *not* be detected by the "classical" methods of measuring photosynthesis and respiration by manometry or chemical analysis; it is only when studies are made using isotopically labelled material that confusion is possible.

Brown (1953), working with an ^{18}O labelled external atmoshpere, showed that the intra-cellular oxygen produced by photosynthesis was used preferentially in respiration, especially when the external oxygen concentration was low. The effect was most marked in the myxophycean *Anabaena* (Brown and Webster, 1953), possibly because in this organism the sites of respiratory oxidase activity were situated particularly near to the sites of photosynthetic oxygen production. If productivity studies were made with labelled external oxygen such behaviour could be interpreted as a "photoinhibition of respiration", but as oxygen isotopes are not likely to be employed for marine productivity measurements the subject is, at present, of little importance.

However, radiocarbon is becoming increasingly popular as a tool for photosynthetic rate studies and the corresponding "photoinhibition of photosynthesis", when photosynthesis is measured by labelled external carbon dioxide, takes on considerable practical significance.

As mentioned earlier, definite evidence for the re-assimilation of intra-cellular carbon dioxide was obtained by Weigl *at al.* (1951), especially at low light intensities and with a low carbon dioxide concentration. Franck (1949) had earlier suggested that photosynthesis could occur from respiratory intermediates and that formation of CO_2 would be stopped at a suitably low light intensity. Ryther (1956b), working with cultures of *Dunaliella euchlora*, heavily labelled with ^{14}C, found evidence for the complete re-assimilation of respiratory carbon dioxide before it left the cells. Nielsen (1955d), on the other hand, disputed this possibility as he was unable to detect any difference in "photoinhibition of photosynthesis" (Nielsen, 1953) when the external carbon dioxide concentration was varied as much as 100-fold. However, Nielsen did find (using *Chlorella* cells heavily labelled with ^{14}C) that some 60% of the carbon dioxide respired was preferentially reused for photosynthesis at an illumination of 8000 lux (i.e. at about optimum light intensity, see Section V).

It may be assumed that the ^{14}C labelled carbon atoms taken into an algal cell are not normally respired or lost by autolysis to any appreciable extent for at least 5 to 10 hours (Weigl *et al.*, 1951; Ryther and Vaccaro, 1954; Ryther, 1956a; Fogg, 1957; Nielsen, 1957a). Therefore, during $^{14}C_2$ uptake measurements of short duration, any intra-cellular respiratory CO_2 will be unlabelled. If, as Ryther has postulated, all this carbon dioxide is utilized for photosynthesis, the rate of $^{14}C_2$ uptake from outside the cell, instead of measuring gross photosynthesis as was at first supposed (Nielsen, 1952a), will measure the *net* rate of photosynthesis down to the compensation point. This follows because:

$$\text{Net photosynthesis} = \text{Gross photosynthesis} - \text{Respiration}$$

The gross rate equals the uptake of both labelled CO_2 and unlabelled *intra*-cellular CO_2 and the respiration term (equal to the rate of production of CO_2) is equal to the rate of uptake of intra-cellular (unlabelled) CO_2. Below the compensation point the respiratory CO_2 can satisfy all demands of photosynthesis and there is no external radiocarbon taken into the cell, although the net photosynthesis is, in fact, negative.

It is difficult to imagine that such a clear-cut state of affairs as that just described can be universally applicable in a complex population of marine phytoplankton. The degree of re-assimilation will surely depend upon the organism, its permeability to carbon dioxide and the extra-cellular concentration of the latter (i.e. the pH of the sea water and the availability of bicarbonate ions, etc.).

If we now consider Nielsen's postulate that only some 50% to 70% (rather than all) of the respiratory carbon dioxide is re-assimilated (Nielsen, 1953, 1955d) the $^{14}CO_2$ uptake rate, instead of being equal to the rate of net photosynthesis, will have some value in between the net and gross rates. In this case, $^{14}CO_2$ uptake will still occur at the true compensation point (equal to some 40% of the gross rate at compensation light intensity) and when no intake of radioisotope can be detected plant cells will be respiring at about twice the rate at which gross photosynthesis is taking place.

Of course, after a prolonged incubation with radioactive carbonate, when all the carbon in the plant is fully labelled with ^{14}C, any further net uptake of radiocarbon is bound to measure net photosynthesis (ref. Nielsen, 1957a) whether re-assimilation takes place or not.

Ryther and Vaccaro (1954) found a fair agreement between $^{14}CO_2$ intake and the *gross* photosynthesis as measured by light- and dark-bottle experiments (see Section IV.E) but the respiration in these experiments was small compared with the gross synthesis. Using a PQ value of 1.25 Ryther (1956a) reported excellent agreement between the *net* rate of photosynthesis by culture of *Dunaliella,* as measured by net oxygen evolution, and the uptake of radiocarbon; the agreement held right down to the compensation point.

There is no real conflict with this general picture, even when the $^{14}CO_2$ intake data of Nielsen and Al Kholy (1956) are considered, if these are taken as reported before the somewhat complex interpretations given to them by the authors; and we may assume with some confidence that experiments with radiocarbon do not measure gross photosynthesis, but a value between gross and net. This value need not always be at the same point in between, although it is probably nearer to the net value.

There is general evidence that healthy algal cells in culture and nearly all phytoplankters in nature respire at a rate somewhere between about 5% and 15% of the rate of gross photosynthesis at optimum light intensity, although the respiration may almost equal the gross rate when the latter is reduced by gross nutrient deficiencies (Barker, 1935; Kok, 1952; Brackett *et al.,* 1953; Ryther, 1954a, 1956a, c; Verduin, 1956a, b; Nielsen and Hansen, 1959; amongst others). For healthy cells under conditions of good illumination, therefore, $^{14}CO_2$ uptake data give about the same result whether interpreted by either Ryther or Nielsen (90% and 94% of gross, respectively, if respiration is 10% of photosynthesis). The differences only become serious when the ratio of photosynthesis to respiration is small.

This ratio can scarcely decrease below 2:1, on the average even in nutrient-defficient warm tropical waters, as otherwise a phytoplankton crop could not survive 24 hours of respiration with only 12 hours of photosynthesis (this point was well taken in the paper by Nielsen and Al Kholy, 1956). With a respiration rate equal to 50% of photosynthesis (a 2:1 ratio), $^{14}CO_2$ uptake data would be measuring 1.0 times net synthesis, or 50% of gross synthesis, according to Ryther, or $100 - \frac{60}{100} \times 50 = 70\%$ of gross (1.4 times net) according to Nielsen. At a 25% respiration rate, which Nielsen (1952a) considers reasonable for the tropics (it may even be high, ref. Nielsen and Hansen, 1959) the values are 1.0 times net and 75% gross (Ryther) as against 1.13 times net or 85% gross (Nielsen). Such differences are scarcely serious, considering the many other uncertainties involved in the measurements of marine phytoplankton productivity, and it is rather surprising that so much controversy should have resulted in the literature. However, the differences do serve to underline difficulties of interpretation which should be resolved as soon as possible.

For the present review we will assume that suitably designed experiments using radiocarbon (see Section IV.F) give a measure between gross and net photosynthesis, and may well vary from one crop of phytoplankton to another. There seems to be no *a priori* reason why the re-assimilation characteristics of various phytoplankters must necessarily be the same. It is possible that $^{14}CO_2$ uptake values are nearer to net than gross photosynthesis but this is far from established as a universal rule for *all* marine photosynthesis.

B. SAMPLING PROBLEMS

Horizontal and vertical variations in phytoplankton productivity are more complex than the corresponding variations in phytoplankton crop because the productivity depends not only upon the crop density but also on the transparency characteristics of the water and the intensity of solar radiation. As will be discussed fully in Section V.C, the *in situ* productivity of the sea can only be measured directly by enclosing small volumes of water within the euphotic zone and studying the rate of plant autosynthesis in each small volume. A more complex, and at present less reliable method, is to remove samples from various depths in the euphotic zone and incubate the endemic populations under some standard radiation conditions, hoping to derive from the data so obtained a measure of the *in situ* behaviour of the total crop.

In either method representative samples must first be removed from the euphotic zone, even if they are later to be returned to the water. We are faced, therefore, with most of the problems that arise when attempting to obtain representative samples for standing crop measurement, problems which have already been discussed in Section III. There are additional complications in productivity work because the inherent productivity potential of a plant population appears to vary in a periodic manner with time (see Section V.B.), necessitating the removal of samples at the same local time each day if comparative results are to be obtained.

Unfortunately, there is evidence that pumping large representative samples from the euphotic zone, which appears to be the most satisfactory answer to the sampling problem for standing crop measurements, may be out question for productivity work. Although Doty (1955) produced some evidence that the vigorous agitation of sea water samples could reduce the photosynthetic activity of the plant crop, it is not clear whether or not the serious inhibition reported by Doty with a pump and 100 m of Tygon hose arose from the action of the pump (presumably pressure reduction effects) or from the metal and Tygon in the system. Clendenning and Brown (1956) found that the centrifugation of *Nostoc* and *Chlorella* at high speed for several hours had no ill effect on the subsequent photosynthetic behaviour of cells if the system were chilled and implied that overheating and anaerobiosis might be the cause of many of the adverse effects reported.

The use of a pump or centrifuge in productivity work has never found favour and Jitts (1957) even went to the length of using the same bottle for collecting samples as for their subsequent incubation, rather than subject the water to any disturbance. However, the subject merits further investigation using an all-plastic centrifugal pump with various types of hosing and impellers. One infers from later work by Doty (1957) that the effect of agitation may have been over-estimated, but the position is not clear.

Conceivably, the results of pressure reduction on the soft-bodied nanno- and ultraplankton of tropical waters (ref. Doty's work) may not be representative of the behaviour of much of the phytoplankton in subarctic regions.

One is left, at the present, with the necessity of using sampling bottles for productivity work, despite the obvious unsuitability of such a procedure. It may be possible to offset some of the errors that result from such unrepresentative sampling by the simultaneous measurement of standing crop and then using the productivity index. This subject will be discussed fully in Section V.A.

For surface sampling, or removing water from the top few meters, large glass bottles, "snatch bottles" of polyethylene, a canvas bucket or a plastic bucket are all quite satisfactory (cf. Riley, 1941a; Miyake *et al.*, 1954; Doty, 1955). When faced with sampling at depth in the euphotic zone, the type of water bottle used assumes prime importance. Metal bottles are out of the question, unless completely lined with inert material (Currie, 1957), because of the adverse effect of even traces of metal ions on the metabolism of most unicellular marine plants.

Quantitative information on the effect of various materials on $^{14}CO_2$ uptake during photosynthesis was obtained by Doty (1955). The use of a brass Nansen bottle, or even of a stainless-steel — nickel bottle with no copper alloys visible, reduced photosynthesis to nearly one-third of its initial rate. Paraffin, lucite, plexiglass and (by definition) glass were without effect but, surprisingly, there was very appreciable inhibition (some 40% to 60%) with Tygon, plywood and Neoprene. From this work it would appear to be a wise precaution to test any new material of construction to be used for bottles, pumps, hoses, filtration equipment, etc., before it is put to use, even if it is seemingly "inert".

Non-metallic water bottles made of glass or plastic have been described by several workers (e.g. Jensen and Nielsen, 1953; Thompson and Chow, 1955; Jitts, 1957) but the bottle suggested by Van Dorn (1956), made of Lucite or Uscolite, appears to have achieved the greatest popularity, mainly on account of its great simplicity and flexibility of design. The bottle can be made with a capacity varying between some 2 to 20 liters and is now used for productivity work at practically all oceanographic institutions in North America.

The separation of zooplankton from phytoplankton before the latter is incubated for productivity studies is essential if a true measure of net primary productivity as distinct from the net production rate (see Section I.A), is required. Such a problem has already been discussed in Section III.A but the present separation could well be cruder and a No. 0 bolting silk net would probably remove most of the zooplankters capable of significant phytoplankton grazing over a period of a few hours. Nielsen and Jensen (1957) claim that filtration through a No. 25 silk net has little effect on the rate of photosynthesis in tropical waters but such a precedure is likely to be of limited applicability (cf. Doty, 1958). For reasons to be discussed later in this section, a separation of bacteria from phytoplankton would be desirable but this is scarcely feasible by any technique of differential filtration. The Millipore Corporation HA membrane filter is probably the coarsest filter that can safely be used if all photosynthetic organisms are to be retained (ref. work described by Nielsen, 1952a; Lasker and Holmes, 1957), and with such a porosity (0.5 micron) few bacteria, even if unattached to plant cells, are likely to pass through the membrane.

There is evidence, at least in lakes (ref. Rodhe, 1957), that nannoplankters photosynthesize more rapidly per unit weight of cells than do the microplankton and it is essential, therefore, that any method of separation or concentration has no injurious effect on these organisms.

C. DIRECT MEASUREMENTS

In principle, any method for measuring standing crop by direct methods (see Section III.B) can be used for productivity measurements if the populations are measured over suitable time intervals and the differences found. Such procedures are extremely tedious and have little to recommend them unless the results are incidental to a detailed survey undertaken for taxonomic purposes. There is the additional disadvantage that counts or volumes have to be converted to combined carbon if results are to have much general applicability.

Several workers have reported productivity measurements by direct means (e.g. McQuate, 1956; Ragotzkie and Pomeroy, 1957; Smayda, 1957) but only in lakes or estuaries. The use of such methods for open ocean studies seems out of the question and direct measurements using cell counts, packed volumes, turbidities, wet weights or algal volumes are mainly appropriate for studying the kinetics of phytoplankton growth in cultures (cf. Ketchum, 1939a, b; Riley, 1943; Edmondson and Edmondson, 1947; Ketchum *et al.*, 1949; Tamiya *et al.*, 1955, etc.).

D. ESTIMATION BY CARBON DIOXIDE CONSUMPTION

The measurement of net photosynthesis by means of the net consumption of carbon dioxide in a known time is direct and, provided that the time interval exceeds a few hours, probably gives a reliable estimate of the mean rate of plant autosynthesis. Difficulties associated with this method are, in the main, experimental.

Estimation of the total carbon dioxide content of fresh or sea water by classical means (acidification followed by gas analysis or the determination of CO_2 volumetrically or gravimetrically) requires great skill if the differences of carbonate content before and after a spell of photosynthesis are to be determined with the necessary precision (cf. Hamm and Thompson, 1941; Saruhashi, 1953). Unlike artifical algal cultures, the concentration of plant cells in natural waters is generally too small for respirometers to be used with any precision over relatively short periods of time. The most rapid and convenient method for measuring carbonate changes in water is to measure the pH and alkalinity of the water before and after an experiment. The productivity of lakes has been measured by such means by several authors (ref. e.g. McQuate, 1956; Verduin, 1956a, b, 1957) but in sea water heavy buffering lessens the sensitivity of such a procedure to a serious extent (cf. Sargent and Hindman, 1943; Verduin, 1956b).

Measurement of the total carbon dioxide content of sea water from the alkalinity and pH is best accomplished from the data and constants derived by Buch and co-workers which have been collected in an easily used tabular form by Harvey (1957b). The second dissociation constant of carbonic acid is now thought to be appreciably different from the Buch values on which the tables by Harvey are based (Lyman, 1957) but it is doubtful whether these changes will alter any calculations based on small *differences* of pH.

A few practical points associated with these measurements merit discussion as the analyst is faced with the difficult problem of measuring small differences in carbonate concentration, differences which will not normally exceed 1% to 2%.

Except under conditions which will be encountered but rarely in temperate seas, the *specific alkalinity* of sea water (total alkalinity divided by chlorinity) may be assumed to be unchanged over a period of a week or less; it is most unlikely that the removal of carbon dioxide as calcium or magnesium carbonate will amount to a significant fraction of the total carbon dioxide used up in phytoplankton production. Slight changes in *total alkalinity* might occur, however, in surface waters if there is intense precipitation or evaporation, so it is best to measure the specific alkalinity at the commencement of a field experiment, using a rapid but not necessarily precise technique for total alkalinity (e.g. Anderson and Robinson, 1946), and then estimate the final alkalinity at the end of a period of photosynthesis by means of a chlorinity determination, using the initial specific alkalinity value. Any change in alkalinity will then be measured by precise chlorinity determinations rather than by the difference between two direct alkalinity results. A direct determination of two such alkalinities to a tenth of a per cent or less, would require considerable skill and be very time-consuming (cf. Cooper, 1934a).

Changes of the total carbon dioxide content of sea water are measured mainly as a change of pH. By graphical interpolation of the data given by Harvey it may be seen that the photosynthetic fixation of 100 mg C/m^3 would change the pH of sea water (Cl = 19‰) at 10° C by about 0.025 of a unit at pH 8.1. Such changes might well be expected in the course of a few hours during a bloom of phytoplankton in subarctic waters. Working with great care and careful attention to temperature control it is possible to record meaningful changes in pH of the order of about 0.005 to 0.01 using modern high amplification battery-operated pH meters, such as the Beckman Model GS meter. A value for the statistical precision possible under good field conditions has not been reported but it is not unreasonable to assume that differences of 0.01 of a unit might well be significant. Experiments *in situ* or by incubation in large glass vessels such as carboys, could therefore be undertaken if a period of at least a day were allowed.

For *in situ* experiments to have any meaning one must assume that the re-establishment of equilibrium between oceanic and atmospheric carbon dioxide is negligible during an experiment. Cooper (1956) has pointed out that the exchange between atmospheric carbon dioxide and the dissolved gas would be expected to proceed much less rapidly than the rate of exchange of atmospheric oxygen. Taking the values given by Redfield (1948) or Adeney (quoted by Harvey, 1957) for oxygen exchange it is obvious that no significant error is made if one assumes that changes in marine carbon dioxide concentration over a period of a few days arise solely from biological causes.

The measurement of net primary productivity by means of carbon dioxide changes has never found popularity, mainly because of the lack of sensitivity, even under favourable analytical conditions, but it would be interesting to see a few more precise investigations attempted in the field. However, changes in the dissolved oxygen content of sea water during photosyntheis, which are of the same order, have received considerable attention. As practically all marine primary productivity, until the present decade, was measured via oxygen determinations the subject merits a review in some detail.

E. ESTIMATION BY OXYGEN EVOLUTION (LIGHT AND DARK BOTTLE)

The measurement of gross photosynthesis that is obtained by following changes in the dissolved oxygen in a water sample, using the Winkler titrimetric method, is commonly referred to as the "Light and Dark Bottle" technique, abbreviated to L and DB (LB, DB, etc.) in the present review.

The first application of such a method in the sea is commonly attributed to Gaarder and Gran (1927) but earlier work is recorded (e.g. Pütter, 1924), and it was shortly thereafter used in both America and Europe (Marshall and Orr, 1928; Gran and Thompson, 1930; Nielsen, 1932). A large number of investigations in eutrophic lakes and sea waters have subsequently been reported (ref. e.g. Clarke and Ostler, 1934; Nielsen, 1937b; Jenkins, 1937; Riley, 1939b; Manning and Juday, 1941; Riley, 1941a, b, 1943; Gessner, 1944; Edmondson and Edmondson, 1947; Riley and Gorgy, 1948; Nielsen, 1951a; Edmondson, 1956; McQuate, 1956; Riley *et al.*, 1956; Verduin, 1956a, 1957; Smayda, 1957, etc). The method was also used by Riley in oligotrophic areas in the tropics (see Riley, 1938a; Riley, 1939b; Riley and Gorgy, 1948).

In brief, the method consists of enclosing a water sample, containing a natural plant population, in glass bottles and exposing the bottles to light, either in the euphotic zone (suspended by wire at the depth from which the samples initially originated) or in some form of incubator on board ship, where either sunlight or artificial light can be used for illumination. In a parallel experiment a portion of the initial sample is held in a darkened bottle for the same length of time and at the same temperature as the illuminated sample. (Details of the design of suitable incubators and of experiments carried out in situ in the euphotic zone will be defererd until Section V.C.).

The initial oxygen content of a water sample is determined by the Winkler method; this initial concentration will be referred to as IB. The difference between this titration and the titration found from water in an illuminated bottle after a suitable period of exposure (LB) gives a measure of the net evolution of oxygen arising from photosynthesis (LB – IB). This difference is *not* necessarily equal to the true net photosynthesis of the plants enclosed in the LB, however, as oxygen may have been consumed by both bacteria and animals in addition to the oxygen used by the respiration of the plant cells proper. It is more common to use the L and DB technique for measuring gross photosynthesis. This is achieved by finding also the difference between the initial (IB) oxygen content of the water and the oxygen remaining in a darkened bottle (IB − DB). Such a difference (a loss of oxygen due to respiration) is assumed equal to the total respiration occurring in the illuminated bottle over the same period of time and thus, if added to the net value obtained from the light bottle alone, gives a measure of the gross photosynthesis from the relationship:

$$\begin{aligned} \text{Gross photosynthesis} &= \text{Net } O_2 \text{ evolved} &&+ O_2 \text{ used for respiration} \\ &= \text{Gain in LB} &&+ \text{Loss in DB} \\ &= \text{LB} - \text{IB} &&+ \text{IB} - \text{DB} \\ &= \text{LB} - \text{DB} \end{aligned}$$

Hence, unless the net oxygen evolution and the respiration need to be estimated separately, the initial water sample does not have to be analysed for its oxygen content although it is good practice to do this as an overall check on the method.

1. MAXIMUM PRECISION POSSIBLE WITH THE LIGHT AND DARK BOTTLE METHOD

It is of considerable importance to estimate the ultimate precision of the L and DB method from purely analytical considerations, apart from the numerous sources of error and uncertainty discussed later in this section. Ryther and Vaccaro (1954) mentioned a figure of 0.1 ml O_2/l as being the smallest significant difference in L and DB work, an estimate which was later reduced somewhat (to 0.05 ml O_2/l by Ryther in his review (Ryther, 1956a). Nielsen (1957a) suggested a limit of 0.02 ml O_2/l if duplicate determinations were made on both LB and DB samples.

In work at the Nanaimo laboratories of the Fisheries Research Board of Canada, the Winkler method, carried out under ideal experimental conditions using either a starch or a "dead stop" electrical end point, gave a standard deviation, σ, of about 0.017 ml O_2/l at a level of about 6 ml O_2/l. The present author is satisfied that this precision cannot be improved upon by any reasonable technique and that under sea-going conditions the σ value will almost certainly deteriorate. However, taking the somewhat idealized value of $\sigma = 0.017$ ml O_2/l and assuming a Gaussian distribution of errors the σ for the *difference* between two oxygen determinations of comparable magnitude can scarcely be less than 0.024 ml O_2/l for single determinations or 0.017 ml O_2/l if we undertake duplicate titrations of both LB and DB. Assuming a PQ of 1.2 and *taking the 0.05 probability limit as a criterion of statistical significance* we find that the smallest amount of photosynthesis that can be measured by the L and DB technique is some 20 mg C/m^3 with single titrations or about 15 mg C/m^3 if duplicate titrations are averaged. The possible spread of any determination is similarly ±20 or ±15 mg C/m^3, respectively. If incubation periods are not to exceed 24 hours we cannot expect to use the "oxygen" method if the rate is below about 20 mg C/m^3/day. It must be stressed that these figures represent an ideal precision, rarely if ever to be expected under field conditions, and take no account of other experimental sources of error. Also it is not clear how the method can be improved significantly and yet have any routine applicability. The difficulty is essentially one of measuring the difference between two large quantities and is common to all techniques. If the polyethylene membrane oxygen electrode described by Carritt and Kanwisher (1959) should prove to have sufficient stability it might be possible to set two of these electrodes in opposition, one in a light bottle and one in a dark bottle, and then amplify any difference so as to record small changes, but no details are yet available.

2. SOME SOURCES OF ERROR IN THE LIGHT AND DARK BOTTLE TECHNIQUE

Apart from variations to be expected from purely analytical sources in the Winkler titration method, many other possible errors can be incurred. Gessner (1944) has given an excellent account of some of these and Riley (1938a, 1941b) has shown his awareness of many of the problems that arise in L and DB experiments. The subject is discussed at length by Nielsen (1957a); see also Pratt and Berkson (1959).

One of the most serious potential sources of trouble is the formation of bubbles in the bottles during incubation. No bubbles appear if bottles are beneath a meter or so of water but they may occur in incubators or in the surface sample of a string of bottles set in the euphotic zone. Nielsen (1957a) has suggested pulling a partial vacuum or mixing samples with a little de-aerated sea water before commencing incubation. It is perhaps better to collect samples first in large containers which are shaken vigorously to remove supersaturation before filling the water into the bottles used for the determinations.

The Winkler method cannot be used with sufficient precision in waters heavily contaminated by sewage or by decaying plant and animal material or even in waters containing excessive phytoplankton. Nielsen implies that a significant iodine consumption can arise from an "iodine value" of phytoplankton oils, but the occurrence of such conditions in a marine environment will be rare.

It must be remembered that a correction for the photosynthetic quotient is necessary before net or gross photosynthesis measurements made via oxygen evolution observations can be compared with the results of other methods. As the PQ values for natural populations are generally uncertain to at least 10% *no better agreement between L and DB experiments and other methods can be expected on this count alone!*

Care must be taken to ensure that the temperature of dark and light bottles are kept to within a few degrees of each other as respiration in the dark bottle will generally be a sensitive function of temperature. The respiration of animals will possibly be greater in a light bottle (ref. Riley, 1938a) but the bulk of the animal population must be removed for satisfactory work (see Section IV.B) and this problem should not arise. It is more important that plant respiration be the same in both LB and DB. As mentioned previously in the present section, the level of respiration of an algal cell should change but little during short intermittent periods of illumination and darkness but over prolonged periods of darkness, such as could be encountered in a DB, the respiration of plant cells almost certainly decreases to a marked extent and the error may be considerable (Ryther *et al.*, 1958).

Respiration may also be somewhat greater in the heavily oxygenated water surrounding a cell during photosynthesis than in the oxygen-depleted water surrounding a cell in the dark (Gessner and Pannier, 1958). For these reasons, other factors constant, the L and DB method would be expected to *underestimate* gross photosynthesis (see also Riley, 1938a; Ryther and Vaccaro, 1954). The effect may partially offset the errors arising from the bacterial respiration discussed later.

Finally we should mention corrections necessary for the growth of algal populations during the course of L and DB experiments. Some increase in cell number or in the total fixed carbon content of plant cells has been reported in dark bottles (e.g. Gaarder and Gran, 1927; Riley, 1938a, 1941a), possibly due to heterotrophic growth, but the effect would be expected to be small in comparison with the undoubted growth in the algal populations of light bottles. In experiments lasting even for one day these cell numbers might well double, whereas a loss of cells by death could occur in the dark bottles (Edmondson and Edmondson, 1947). Pratt and Berkson (1959) have shown the rate of plant growth in a LB can be greater than would be the case in nature, due to some form of "wall effect" in the bottle stimulating growth via bacterial activity. The main error thus introduced is from the assumption that a DB respiration correction, measured on the initial crop, applies to the light bottle in which the crop may have increased substantially.[2] Thus another error is incurred which tends to lead to an *underestimate* of gross photosynthesis. No quantitative correction is possible unless an initial estimate of plant crop is made. The effect will be greater, the greater the productivity of the water and the larger the ratio of respiration to photosynthesis, but it is unlikely that the error will exceed some 5 to 10% of the total synthesis.

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[2] It must be remembered that the mean rate of photosynthesis measured by the L and DB method (or other techniques) is not the same as the true rate of photosynthesis measured by the differential $\frac{dp}{dt}$. If p_t is the amount of fixed carbon at time t and p_o the amount at a zero reference time, t_o, then the rate as measured experimentally is:

$$\frac{p_t - p_o}{t} = \frac{D}{t}$$

where D is the increase of carbon found in the LB. If plant growth is approximately exponential the true rate at time t_o is given by the expression:

$$\frac{dp}{dt} = \frac{p_o}{t} \times 2.3.\log\left(\frac{D + p_o}{p_o}\right)$$

which necessitates a knowledge of p_o for its calculation. p_o is rarely known.

If the fractional increase of plant material does not exceed about 20% during an experiment the true rate at zero time, $\frac{dp}{dt}$ is equal to $\frac{D}{t}$ to within a few per cent. The true rate at a time $\frac{t - t_o}{2}$ however, is still close to the value for $\frac{D}{t}$ when D is as great as p_o (that is, when the population has doubled). For most experiments with marine phytoplankton the time of an experiment should therefore not exceed about 2-3 hours if we wish to find $\frac{dp}{dt}$ at t_o, or 10 hours for an estimate of $\frac{dp}{dt}$ at time $\frac{t - t_o}{2}$.

Needless to say dark bottles must be made completely light tight. Paint is unsatisfactory. One or two thicknesses of black "Scotch Tape" on the bottle, with aluminum foil pressed around stopper, is generally found to be satisfactory.

3. Errors Arising from the Presence of Bacteria

The most serious criticisms that have been levelled against the L and DB technique have been directed against its use in oligotrophic areas and arise mainly from E. Steemann Nielsen.

G. A. Riley, in his work in the Sargasso and Dry Tortugas, found very variable rates of gross photosynthesis but many values rated around about 50 mg C/m^3/day. The values found by carbon-14 data in tropical waters (Nielsen, 1952a) were less than 10% (often less than 5%) of these and considerable controversy has arisen about the cause of this discrepancy.

Riley (1953) stressed the great variability of productivity in tropical seas both in time and location and rightly cautioned against too hasty a judgment before more extensive data were available. However the bulk of carbon-14 measurements now substantiate earlier results (see later in this section) and Nielsen (1954) reports parallel experiments in the Sargasso area that give L and DB results very much greater than those obtained from $^{14}CO_2$ uptake.

The trouble cannot be resolved by assumptions as to the significance of $^{14}CO_2$ measurements. Even if the radiocarbon technique were measuring only net synthesis this could not be less than some 50% of the mean gross value were a plant population to survive over a 24-hour period (Nielsen and Al Kholy, 1956). Whatever is being measured by the uptake of $^{14}CO_2$ it can scarcely be less than a half to a third of the true gross photosynthesis value and we have to look to other factors to explain L and DB results well over 10 times as great. Some of the discrepancy may well be attributable to chemical errors in measuring the extremely small oxygen differences found in oligotrophic waters, even after incubating for several days. Results as reported by both Riley and Nielsen presuppose a precision of Winkler determinations unobtainable by most trained chemists. Low $^{14}CO_2$ uptake values could also arise from carbamino-carbonate uptake (see later) but we have no idea of the magnitude of such an effect in tropical oceanic areas.

The gist of the criticisms levelled by Nielsen is that the L and DB technique, when used in oligotrophic waters, gives a falsely high estimate of respiration as measured in darkened bottles. The bulk of this respiration (and much of the supposed gross photosynthesis actually arises from the DB correction) is not, according to Nielsen, actually occurring simultaneously in the light bottles. It must be reported, in fairness, that sometimes the LB increase alone exceeded $^{14}CO_2$ uptake values (Riley, 1953) but we are dealing with figures very near to the limit of experimental precision.

It is well known that the bacteria in water samples tend to proliferate directly the water is enclosed in a small container or whenever else a solid surface is presented to the population (ref. e.g. Zobell and Anderson, 1936; Zobell, 1943). It is to be expected, therefore, from the nature of L and DB experiments, that a bacterial respiration factor will assume considerable importance. However, provided that the bacteria grow equally in both LB and DB this should introduce no systematic errors.

It was Nielsen's (1952a, b) contention that the results obtained by Riley were too high because bacterial respiration was much greater in the dark bottles than in the light bottles, the population in the former having developed much faster than in the latter. This "bactericidal effect" in the light bottles was first attributed to the action of light itself, but this has subsequently been shown to be impossible (Riley, 1953b; Vaccaro and Ryther, 1954). Nielsen never disputed results obtained by the L and DB method in eutrophic seas (1952a, b) but he insisted that a difference in bacterial growth rate occurs with oligotrophic water. This he now attributes mainly to a chemical bactericidal agent manufactured by growing algae under the influence of light. Such agents have been reported as formed by *Nitzschia* and *Chlorella* (Wakesman *et al.*, 1937; Pratt *et al.*, 1944). Nielsen (1955c) demonstrated indirectly a possible bactericidal effect with *Chlorella* and a marine *Thalassiosira* by showing that the presence of these algae inhibited the total respiration in a light bottle. Vaccaro and Ryther (1954), on the other hand, were unable to detect any effect of light on an inoculum of bacteria in light and darkened bottles, with or without natural plankton populations present. Nielsen (1957a) has implied that this was due to the fact that the natural eutrophic water used in their experiments already contained a large amount of inhibiting agent. In tropical waters, according to Nielsen, sufficient local concentrations of such inhibitors can only be formed by the algae whilst actually growing under illumination. Therefore bacteria proliferate in the dark bottles where their growth is also encouraged by the presence of dead or dying plant cells (see also Nielsen, 1955b). However, some caution is necessary here as Moscovits (1958) has shown conclusively that the growth rate of a marine *Pseudomonas* is actually *increased* by the presence of an illuminated *Nitzschia* culture and no bactericidal effects could be detected by Jones *et al.*, (1958) in tropical waters.

Whatever the cause of the discrepancies between L and DB results and those obtained by radiocarbon there can be little doubt but that the former method is suspect and not particularly suited for use in oligotrophic waters. An independent check on radiocarbon results is still very desirable, however, and a method giving gross primary productivity would be particularly interesting in tropical oceans.

It is possible that quite reliable results could be obtained by the L and DB technique if the crop were first concentrated some 10-fold before incubation but workers have been reluctant to use such an approach, presumably for fear of upsetting the natural metabolism of relatively "delicate" tropical phytoplankton. Much would depend upon the technique used and there seems no reason why investigations along these lines should not be attempted. If the contentions of Steemann Nielsen are substantiated it is difficult to see why dense cultures of, say, *Chlorella* should not be maintained to give water containing a high concentration of bactericidal agent, a little of which could be added to oligotrophic samples before incubation. Presumably the L and DB experiments might then behave in much the same way as if the samples consisted of eutrophic water, except that prolonged incubation periods would be necessary. Another approach which might merit preliminary trials would be to inhibit photosynthesis by chemical means so that both bottles could be kept in the light. Rabinowitch (1945, 1951, 1956) has presented a very full review of the inhibition of both photosynthesis and respiration by chemical agents. Cyanide, sulphide, phenylurethane, 2, 4-dinitrophenol, *o*-phenanthroline and hydroxylamine all suppress photosynthesis in algae when present in solution at a concentration of some 10^{-4} M or less. Sodium azide is particularly powerful (Gaffron, 1944). Most suppressors of photosynthesis first enhance respiration and then, as the concentration of inhibitor is raised, the respiration of plant cells returns to a normal rate before finally being reduced. Concentrations are rather critical and doubtless depend upon the species of algae under observation. Hydroxylamine, at about 10^{-4} M concentration, may be one of the best reagents to use for suppressing photosynthesis without altering respiration (Gaffron, 1939) although some bactericidal effect might be expected. 2,4-dinitrophenol at a concentration of 5×10^{-5} M has little effect on the respiration rate of *Chlorella* or *Navicula* over relatively short periods of time whilst completely suppressing photosynthesis (Holzer, 1954; Lewin, 1955b). The suppression of photosynthesis by quinone is described by Miyachi *et al.* (1955).

F. ESTIMATION FROM THE RATE OF CARBON-14 UPTAKE

The use of radioactive carbon to measure the uptake of carbon dioxide by phytoplankton was inevitable once the ^{14}C isotope became readily available, but the credit for its first application to marine productivity studies goes to Nielsen (1951b), who established a general shipboard technique (1952a) that has been changed very little by subsequent workers. The comparative simplicity of the procedure and, above all, its sensitivity, has led to the widespread use of radiocarbon for measuring primary productivity, both in the sea and in lakes (ref. e.g. Nielsen, 1952a; 1954; 1955a; Doty, 1955; Ryther and Vaccaro, 1954; Miyake *et al.*, 1954; Doty and Oguri, 1956; Rohde, 1956, 1957; Holmes *et al.*, 1957; King *et al.*, 1957; Holmes, 1957; Nielsen, 1957c; Nielsen and Jensen, 1957; Berge, 1957; Jitts, 1957; Wimpenny, 1957; Corlett, 1957; Cushing, 1957c; Currie, 1957; Steel, 1957a; Ryther and Yentsch, 1958, and many others).

The interpretation of results obtained by the ^{14}C method is, unfortunately, not so simple as the technique itself and it is perhaps regrettable that some of the enthusiasm and effort initially directed towards amassing data was not diverted towards a critical evaluation of the method being used. A discussion of some aspects of the problem has already been given in an earlier part of the present section in this review, the part devoted to the interaction of respiration and photosynthesis. It will be remembered that the balance of evidence points to the fact that $^{14}CO_2$ uptake experiments generally give a measure of photosynthesis that is somewhere between the net and gross value, possibly nearer to the former. There are, however, certain aspects of the use of isotopic carbon that require further examination.

1. ISOTOPIC EXCHANGE AND DISCRIMINATION

If plants, the organic matter of which contains only one isotope of carbon, are placed in an environment containing a different carbon isotope (as carbon dioxide) then some exchange between the two isotopic forms is to be expected apart from any transfer that would normally be attributed to either photosynthesis or respiration. Van Norman and Brown (1952) reported a very rapid exchange, which exceeded the respiration rate of the cells being studied. According to Weigl *et al.* (1951) the exchange may amount to only a few per cent of the photosynthetic rate under good illumination conditions but the effect cannot be neglected. $^{14}CO_2$ will be taken into plant cells, apart from any true photosynthesis, and a "blank" correction is necessary. This blank correction is generally made by carrying out a "dark bottle" experiment, as in the oxygen L and DB technique previously described. The DB "blank" gives a fractional correction which is roughly independent of the number of cells present and which generally amounts to 1% or so of the rate of photosynthesis measured under optimum conditions (Nielsen, 1952a; Ryther and Vaccaro, 1954). However, the correction assumes greater importance in some areas, Jones *et al.* (1959) reporting a dark uptake or exchange of 20% or more (see also Doty, 1958). It will also be important with work at low light intensities and if much of a bacterial population is present with the algae. Isotopic exchange with bacteria can be significant in polluted waters (Nielsen, 1957a; Currie, 1957) but is generally not responsible for more than about 10% of the dark $^{14}CO_2$ uptake. (Jones *et al.*, 1959). It would seem best, therefore, to follow the precedent set by Doty and other workers of putting on a routine DB blank with every $^{14}CO_2$ uptake experiment. The slight lengthening of the method is worthwhile in view of the improved accuracy to be expected. Although differences in behaviour of bacteria and algae in the LB and DB may introduce an error in the DB correction the effect is probably second order over the short duration of most radiocarbon experiments.

There has been surprising difficulty in deciding what isotopic discrimination factor should be applied to $^{14}CO_2$ uptake experiments to allow for the fact that the rate of primary carboxylation, etc., by the heavier carbon isotope is somewhat slower than the rate found with the normal carbon-12 isotope. It would be expected from the behaviour of ^{13}C, which has a well-established discrimination of some 2 to 3%, that $^{14}CO_2$ would be fixed by plants at about 95% of the rate found with ordinary carbon dioxide. However, rates nearer to 85% were reported by Weigl *et al.* (1951) and Van Norman and Brown (1952), which would seem to argue for an impossibly large number of isotopic fractionating steps during photosynthesis. It was recognized that not all of this apparent discrimination would arise from purely isotopic effects and the interpretation of both experiments were complicated by re-assimilation and isotopic exchange phenomena. The subject is well reviewed by Nielsen (1955d), who produced evidence for an isotopic discrimination of only 5 to 6%. This would seem to be a common-sense figure and has been used by several workers. The rejection of any correction (e.g. Jitts, 1957), in the absence of a more convincing determination of the true value, appears to the present reviewer to be unnecessarily cautious. Although the position is far from clear, the use of a factor of 1.05 can scarcely fail to improve the accuracy of results, the true factor being in all likelihood within the range 1.03 to 1.08.

Recently Holm-Hansen *et al.* (1958) have provided much-needed confirmation that the presence of a radioactive carbon isotope, *per se,* has no short term adverse effect on the metabolism of a plant which might invalidate the use of ^{14}C as a tracer.

2. GENERAL TECHNIQUE

A detailed description of the carbon-14 technique used in shipboard productivity experiments is given by Nielsen (1952a), Doty (1955), King *et al.* (1957), Jitts (1957), Nielsen and Jensen (1957) and several other workers. The following account summarizes what appear to be the most acceptable modern procedures.

Net primary productivity is unlikely to be less than 1 mg C/m³/day in oligotrophic areas or to exceed a few hundred mg C/m³/day in fertile coastal seas. We are therefore faced with the problem of detecting the uptake of between about 0.01 and 2% of the total carbon in a sample of sea water, if experiments are not to exceed 5 to 10 hours duration. Assuming an overall counting efficiency of about 50% and 200 to 300 counts per minute (cpm) to be the lowest permissible counting rate (see later), then the minimum addition of radioactive carbon for work in oligotrophic seas is some 10 microcuries (μC). This addition is independent of the volume of water being studied if all this water is filtered. There is clearly no upper limit to the amount of radioactive carbonate that may be introduced, except as is dictated by economy, coincidence errors in counting and health and contamination hazards. In general, there would seem to be little point in using more than 50 μC of activity in an experiment and addition of only 1 to 2 μC would be preferable. As a working formula, if E is the percentage efficiency of the counting assembly used, U the anticipated uptake of carbon in mg C/m^3/hour (say a tenth of the daily figure) and N is the desired number of counts per minute from the radioactive phytoplankton, after an incubation period of T hours, then:

$$\mu\text{C carbon-14 to be added} = \frac{N}{E \times U \times T} \qquad \ldots\ldots 1$$

Similarly a rough estimate can be made of the incubation time necessary to give a certain count after adding a fixed amount of radiocarbon.

If the radioactive carbon is added as 1 ml of a carrier-free solution to 200 to 500 ml of a sea water sample there is negligible change brought about in the composition of the sea water, even if the ^{14}C solution is made up in distilled water. There seems little point in using sea water to make up radiocarbon standards as was initially done, certainly a sodium chloride solution would prove an adequate substitute. As there are practical advantages in having the radioactive solution denser than the sea water sample to which it is added a 4 to 5% sodium chloride solution is indicated.

It is now universal practice to prepare 1- to 2-ml aliquots of a standard carbon-14 carbonate solution, each aliquot being of an exact volume and containing a known activity between 1 and 50 μC. The aliquots are stored in small glass ampules. Great care must be taken to ensure that all carbon-14 solutions are stored under aseptic conditions to prevent any loss of $^{14}CO_2$ by organic combination during storage. Ampules, after sealing off, are thoroughly sterilized by autoclaving and if this is done under a dye solution any faulty sealing of the ampules can be detected as dye is sucked into leaky tubes.

A small hypodermic needle is used to transfer the active carbonate solution into the sea water samples prior to incubation. This aliquot is added to the bottom of a bottle containing the sample, the hypodermic is rinsed once with water taken from half way up the bottle, and finally a little water from the top of the bottle is removed for rinsing the ampule. The bottle is securely stoppered and shaken. The presence of a few air bubbles has no harmful effect (unlike the L and DB oxygen method).

After a suitable incubation period the entire contents of the sample bottle are filtered through a membrane filter of 0.5- to 1-micron pore size. Recent work by Lasker and Holmes (1957) indicates that losses of soft-bodied ultraplankton may be serious unless an 0.5-micron filter, such as the Millipore HA filter, is used. The bottle and filter are rinsed with a little filtered inactive sea water (a 3.5% sodium chloride solution is doubtless as satisfactory) and any inorganic carbonate activity is removed from the filtered phytoplankton by exposing the membrane filter to hydrogen chloride vapour or washing it with 10 to 20 ml of an 0.001 to 0.002 N hydrochloric acid solution in 3.5% sodium chloride[3]. The filter is given a final wash with neutral electrolyte to remove all acid and is then dried as rapidly as possible over silica gel in a desiccator, with some form of clamp over each filter to prevent shrinking and distortion on drying. It may be best to put a carbon dioxide absorber in the desiccator to ensure that samples are kept for most of the time in a carbon dioxide-free atmosphere and hence minimize possible exchange of active and inactive carbon dioxide. As there is some evidence that losses of activity may occur if filters containing radioactive plankton are stored for too long it is advisable to count samples within a few months of filtering off the "labelled" algae. Bottles and other equipment must be cleaned frequently with 50% (v/v) hydrochloric acid to prevent the accumulation of activity and should be cleaned thoroughly by detergent every few weeks to guard against the accumulation of bacteria on the walls.

The total carbonate-carbon content of the sea water under study (generally around 25,000 mg C/m^3) is readily calculated from pH and alkalinity measurements (pH and salinity will generally suffice) by the method given by Harvey (1957b); see Section IV D of this review. Let this be W mg C/m^3. If the total cpm of the radiocarbon solution added to the bottle is Z and the cpm given by the filtered phytoplankton is Y (corrected for DB uptake), then the carbon fixed by photosynthesis is taken to be:

$$1.05 \times \frac{Y}{Z} \times W \quad (\text{mg C/m}^3)$$

where 1.05 is a correction put in to allow for isotopic discrimination. To ensure that no loss of photosynthesized radio carbon occurs from plant cells experiments must not exceed about 5–6 hours in duration (ref. Section IV A). It should be remembered that the footnote concerning the approximation of LB measurements to true rates, which was given earlier in this Section in connection with oxygen uptake, applies equally well to the radiocarbon method.

3. SOME RADIOCHEMICAL CONSIDERATIONS

The radiochemical techniques involved in working with carbon-14 are somewhat complicated by the fact that this isotope emits only a very soft β radiation (0.16 MeV) but the subject is fully covered by standard texts on radiochemistry, in particular the treatise by Calvin *et al.* (1949) on radiocarbon. A few notes on some of the more important considerations will be given here for reference.

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[3] This acid wash is now established practice and its need does not seem to be disputed. However, work in the author's laboratory has indicated that quite serious losses of activity can occur with some samples, the losses increasing the larger the volume of acid used and the slower the filtration rate. As the amount of *inorganic* carbon fixed by photosynthesis can scarcely be more than a trivial fraction of the total carbon uptake over a short period, even with coccolithophores, there seems some doubt as to whether this acid treatment is worthwhile. The errors involved by its neglect may well be less than those brought about by its use. The original use of hydrochloric acid vapour by Nielsen (1952a) is not open to this criticism but still may be unnecessary for the majority of marine samples.

The standard deviation of any radiochemical determination is limited, in the last analysis, by the properties of radioactive atoms, as the coefficient of variation cannot be less than:

$$\frac{100}{(N \times t)^{1/2}} \quad \ldots\ldots 2$$

where N is the number of counts per minute (proportional to the concentration of radioactive substance) and t is the time, in minutes, during which the counting takes place (i.e., $N \times t$ is the total number of counts recorded). The coefficient of variation thus obtained may be used in Gaussian statistics and therefore, for an 0.05 probability criterion (19:1 odds in favour), results can be no better than, say, ±2% when 10,000 counts are recorded. If only some 2000 counts are made, as seems to be common practice in productivity work, the limit of uncertainty from purely radioactive considerations is raised to ±4.5%. Although Nielsen (1952a) and others claim that no purpose is to be gained by working to a better precision than this, the present writer can see little point in introducing uncertainties of this magnitude when the precision is so easily improved by increasing the counting time or the initial activity. For practical reasons in routine work involving many samples it is undesirable to have a counting time exceeding about 5 to 10 minutes. The activity of precipitates should, therefore, exceed 200 to 300 cpm and preferably be 1000 cpm or greater. If the counting rate decreases below some 100 to 200 cpm additional counting time is required to offset errors introduced by variations in background radiation. As most Geiger systems introduce appreciable "coincidence errors" at counting rates exceeding about 5000 cpm this should be taken as the upper acceptable level unless a coincidence correction curve is determined for the equipment used.

Miyake *et al.* (1954) report experiments in which the "labelled" plankton is burnt and counted *via* the radioactive carbon dioxide gas that is produced, but the technique now universally employed is to count the plankton on a 1-inch diameter membrane filter by an end-window Geiger tube or in a gas flow counter working in the Geiger range. The use of a flow counter increases the sensitivity of counting some 10- to 20-fold over an end-window assembly (30 to 50% as against 2 to 5%) but end-window counting has some advantages in simplicity and ease of decontamination. A closed Geiger counter is not adversely affected by traces of water or volatile substances in the sample.

Each sample count should be corrected for coincidence error and background and adjusted for Geiger efficiency by frequently counting a standard source. However, the main difficulty in the radiochemistry involved in carbon-14 productivity work is the calibration of the stock radiocarbon solution added to each sample (the term Z in the expression $1.05 \times \frac{Y}{Z} \times W$; see page 85).

We are then faced with complications arising from the self-absorption and scattering of β particles.

The β rays from ^{14}C are both absorbed and scattered. Absorption is mainly a function of the "thickness" of the absorber (defined in radiochemical work as a mass per unit area, generally mg/cm^2, and is nearly independent of the type of matter comprising the absorbing material. Because of a near-cancellation of several effects the final absorption pattern of the β particles is approximately exponential with the thickness of absorber, in a manner reminiscent of the attenuation of light through a uniform medium. It is clear, therefore, that a precipitate containing radiocarbon will partly absorb radiation from the ^{14}C atoms in the precipitate itself, giving rise to the phenomenon of "self absorption". If a_g° is the activity (cpm) per unit thickness of a precipitate at zero thickness and a_g is the observed activity per unit thickness when the thickness is g mg/cm^2, the relationship:

$$a_g = a_g^\circ \times \frac{1 - \exp(-\eta.g)}{\eta.g}$$

or if $\eta.g$ is small:

$$a_g = a_g^\circ \times \exp(-\eta.g) \qquad \ldots\ldots 3$$

holds to a good approximation with values of g between about 5 to 10 mg/cm^2 down to 0.5 to 1 mg/cm^2. "η" is a constant known as the self absorption coefficient. This equation assumes that the specific activity (number of radioactive atoms per unit thickness) is the same for all precipitates.

There is now evidence that η is in fact not truly constant and increases as g decreases. The relation of a_g to g may be more nearly hyperbolic at low thicknesses (Hendler, 1959).

Unlike the absorption of β rays, the scattering of β rays depends upon both the nature and thickness of the material causing scatter. When membrane filters are mounted on metal dishes, trays or "planchettes" for counting purposes the β rays ejected in a direction away from the Geiger tube are partly scattered back into the tube by the material making up the filter and its mounting. The proportion of rays back-scattered increases as the thickness of the mount increases until this thickness exceeds some 6 to 8 mg/cm^2 when the back scattering reaches a constant value. This value, however, depends upon the atomic number of the material backing the sample and is 2 or 3 times as great with, say, a copper planchette as with an aluminum mount. There is also scatter from the membrane filter (some 5 mg/cm^2 thickness) and from the precipitate being counted.

These scattering effects, and the geometry of the counting assembly used, make it impossible to generalize any absolute value for η in equation 3 or to extrapolate with complete confidence to zero thickness.

The phytoplankton containing ^{14}C, filtered from 200 to 500 ml of sea water onto a 1-inch diameter filter, will rarely contribute a "thickness" exceeding 0.1 mg/cm^2 and, provided that filters are uniformly dry, self absorption effects from the plankton may generally be neglected. Matters are assisted by the fact that particles scarcely penetrate the pores of a membrane filter of the Millipore type but stay within a few microns of the surface. Scattering and geometrical effects are very constant unless much extraneous solid matter is present, such as sand, when low results could occur. (This possibility must be borne in mind when working in shallow inshore areas or where the standing crop of phytoplankton much exceeds 300 mg C/m^3.)

If we wish to standardize our initial radiocarbon solution prior to a photosynthesis experiment, however, certain difficulties arise. These may be overcome in two ways.

We may take a suitably small aliquot of the radioactive solution (1% or less of the volume in an ampule) and attempt to spread it, with or without dilution, uniformly over the surface of a membrane filter or a sheet of carbonaceous material of similar thickness. The solution used must be evaporated to dryness without loss of $^{14}CO_2$ by exchange with atmospheric $^{12}CO_2$ and must introduce less than a few tenths of a milligram of total solids. Such a procedure is more difficult than might be supposed and it is therefore more general to precipitate the active carbonate with an excess of inactive barium carbonate, $Ba^{12}CO_3$, and count the precipitate. Aliquots of the ^{14}C standard solution are mixed with various amounts of inert carbonate, as sodium carbonate, and precipitated with barium hydroxide containing a little ammonium chloride to lessen co-precipitation of barium. The precipitates are dried and weighed to give the thickness in mg/cm^2. It is then general practice to plot the values found for counts per minute against g on semi-log paper and extrapolate to $g \rightarrow 0$, normalizing the value found to make the cpm value at zero thickness equal to unity or to 100%. The curve so obtained is assumed to apply for a given apparatus. It will therefore apply to the observed count from a known thickness of barium carbonate precipitate derived from a known volume of standard carbon-14 solution. The count at zero thickness can thus be evaluated.

There are, however, objections to this linear extrapolation procedure unless the experimental data are extended to thicknesses well below 0.5 to 1 mg/cm^2. Unlike phytoplankton, barium carbonate has scattering properties that are greater than those of either the membrane filter or materials such as Al, Ni, Cu, Fe, etc., that are normally used to make the mounting planchettes. In such circumstances semi-log activity plots can have slight maxima at small thickness values. To obtain experimental points in the low thickness range a uniform slurry of $BaCO_3$, containing an appropriate activity, may be standardized by noting the activity and weight of a large aliquot. Progressively smaller aliquots are then filtered off and the assumption is made that the amounts of radioactive material and the thickness of precipitates are proportional to the volumes taken. As the specific activity of all precipitates is the same, an a_g vs. g curve can be constructed to give an extrapolated a_g° value. At very small thicknesses there is some indication that a linear plot may give a better extrapolation than a semi-log plot because of the breakdown of the exponential relationship mentioned earlier.

If we remember that the presence of even 1 mg/cm^2 of precipitate can reduce the count from a given amount of ^{14}C by 10-30% it will be seen that the correct evaluation of the zero-thickness counting rate is of more than academic concern. Errors in ^{14}C standardization give proportional errors in all productivity measurements. These errors are constant for all the work carried out with a given piece of equipment and thus *relative* values are not in question but the comparison of data between one institution and another can be affected to an appreciable extent. In comparison with other possible errors in productivity measurements this may seem trivial but, again, the present author sees little point in the accumulation of inaccuracies that can be avoided with a little care.

4. PRECISION, ACCURACY AND SENSITIVITY

With careful blank control, standardization of ^{14}C solutions and the correct evaluation of the inactive carbonate in sea water samples the systematic errors in $^{14}CO_2$ productivity work should not exceed some 5%. The precision of methods will depend to some extent upon the radiochemical technique used, especially the number of counts recorded and the corrections for background. There seems no reason why the precision (95% criterion) should not be kept to ±5% or better but the precision of many stages in the method (addition of ^{14}C solution, filtration, incubation, etc.) does not appear to have been evaluated.

Nielsen (1952a) gives coefficients of variation for a split sample of sea water that are around some 5-8% but it is not clear to what extent uncertainties from counting statistics enter these estimates. Doty (1957) reports a coefficient of around 11% and a fall off in precision when the productivity is less than 0.1 mg C/m^3/hr. The limit of detection of 0.02 mg C/m^3 suggested by Ryther (1956a) would seem to be rather idealized. The detection limit is presumably governed by the variability of DB blank counts. From the blank determinations reported by Ryther and Vaccaro (1954) one would assume this limit to be of the order of 0.1 to 1 mg C/m^3 but no statistical data has been reported which enables such a limit to be calculated with any certainty. The carbon-14 method is certainly capable of some 50 to 100 times the sensitivity of the L and DB oxygen method under similar routine conditions.

A serious error in the radiocarbon technique could arise if a substantial fraction of the carbonate taken from the water by plants were in the form of a carbamino complex (ref. page 63). Added $^{14}CO_2$ equilibrates with such complexes very slowly (Smith, 1958) and hence low values for photosynthesis would be recorded by the uptake of labelled bicarbonate. The possible magnitude of such errors is not known but in waters high in organic material it could perhaps be significant, especially with experiments of short duration. The number of direct comparisons of $^{14}CO_2$ and L and DB data in nature are regrettably few but often the $^{14}CO_2$ uptake results are substantially below the net results calculated from oxygen evolution (J. H. Oliver, private communication, 1958; Ryther and Yentsch, 1958; W. H. Thomas, private commuication, 1959). Work in progress at the Nanaimo laboratories of the Fisheries Research Board of Canada is confirming these observations.

G. METHODS BASED ON PIGMENT CONTENT

As chlorophyll *a* is an essential catalyst for photosynthesis it might appear reasonable to assume that the gross rate of photosynthesis taking place in sea water illuminated by light of a given intensity would be a function of the chlorophyll *a* content of the sea water. Furthermore, at optimal light intensity, or at intensities below the optimum, the relationship might be expected to be linear with respect to both chlorophyll content and light energy.

It is not surprising, therefore, that such a relationship has been sought after by many workers and has been demonstrated to exist, at least in an approximate fashion. Examples of this work will be found in papers by Emerson (1929), Fleischer (1935), Manning *et al.* (1938), Emerson *et al.* (1940), Sargent (1940), Riley (1941a, b), Manning and Juday (1941), Gessner (1943, 1949), Edmondson

TABLE VIII. Relation of gross photosynthesis to pigment content.

Approximate mg C/hour synthesised per mg chlorophyll at optimum light	Reference	Remarks
3	Gessner, 1949	Mean value for natural lake populations
1–2	Holmes, 1957	Incubator values for natural marine populations
1–6.5	Clendenning *et al.*, 1956	Cultures of Chlorophyceae and Myxophyceae
ca. 4.5	Currie, 1957	Maximum in euphotic zone; all pigments added together
ca. 4.5	Edmondson, 1956	Assuming a tenth of daily value at *ca.* 0.13 ly/min
ca. 1	Fleischer, 1935	Light intensity uncertain but probably slightly greater than optimal. *Chlorella* cultures
4–6	Gessner, 1943	Lakes
2	Manning and Juday, 1941	Lakes
3	Riley, 1941b	Coastal water
6	Ryther and Yentsch, 1957	Average for coastal waters
5–10	Ryther and Yentsch, 1957	Various pure cultures
3	Ryther, 1956a	Culture of *Dunaliella euchlora*
4.5	Shimada, 1958	*Net* photosynthesis at a light intensity probably sub-optimal

(1956), Clendenning *et al.* (1956), Ryther (1956a), Holmes (1957), Currie (1957), Ryther and Yentsch (1957), Yentsch and Ryther (1957), Ryther and Yentsch (1958), and some of these data are summarized in Table VIII. (See also Odum *et al.*, 1959, for some *in situ* estimates under natural conditions of illumination.)

The raw data in the literature are presented for such a variety of algal species, conditions of illumination and temperature, environment, nutrition, etc., that it is difficult to evaluate a self consistent set of factors. The results in Table VIII are very approximate as they have to be adjusted to give a standard unit of productivity at a standard illumination, the latter being, as near as can be judged, the optimum illumination for maximum photosynthesis. Much of the variation, however, is inherent in the original data. The range is at least an order of magnitude, between about 1 and 10 mg C/hr for each milligram of chlorophyll, when the illumination is around 0.1 ly/min of photosynthetically active radiation. The data average out to about 4 mg C/hr and this figure gives rise to the equation reported in Section I. B.2. Results obtained by using this equation approximate to those obtained by using the expression given by Ryther and Yentsch (1957). The factor of 3.7 mg C/hr suggested by these authors, however, implies a precision which can scarcely be intended.

The use of a simple equation to relate chlorophyll and light data to gross primary productivity is an attractive concept, even although net primary productivity is often of more practical interest. In favourable circumstances the prediction of gross photosynthesis may be within 20% (cf. Ryther and Yentsch, 1957) but a variation as great as 2- or 3-fold is more likely and it is worthwhile to enquire why a precise relationship is not to be expected.

The building up of "assimilatory power" by reserves of ATP and $TPNH_2$ (ref. e.g. Arnon *et al.*, 1958; Trebst *et al.*, 1958) may be quantitatively similar in all algal cells, as it takes place primarily in the grana of the chloroplasts by enzymes located along with the plant pigments, but there is no obvious reason why the rest of the fixation of carbon dioxide should not be greatly dependent upon the class, genera or even species of alga under study (ref. the remarks by Rodhe, 1957, on nannoplankton). One would also expect the prehistory of nutrition, temperature and illumination to be important and there is every evidence that this is indeed the case (ref. Emerson *et al.*, 1940; Sargent, 1940; Clendenning *et al.*, 1956; Ryther, 1956a; Yentsch and Ryther, 1957) A notable example of this is found when chlorosis is introduced by magnesium deficiency, rather than by the more normal procedure of starving cells of iron or nitrogen. With magnesium deficiency (Fleischer, 1935; Haskin, 1941) the gross photosynthesis rate remains low and nearly constant despite changes in the chlorophyll content of *Chlorella* cells. However, variations from all these sources may not amount to a great deal and the factor for a mixed population under naturally occurring conditions could well be constant to within ±25% or better for a given location, although varying by a factor of 4 or more between eutrophic and oligotrophic environments (ref. e.g. Ichimura, 1958).

Apart from any physiological considerations, the ratio of chloroplast volume to total cell volume would be expected to affect the relationship. Diffusion kinetics predict that the uptake of carbon in a given time by a large organism containing relatively little chlorophyll would likely be less, per unit chlorophyll and per unit carbon, than for a small organism with a greater pigment content. Experimental evidence for this has been reported by Wright (1959). The nature of the distribution of chloroplasts within an organism must also be relevant.

A more serious practical source of uncertainty is associated with the correct estimation of chlorophyll. We should, ideally, record only that chlorophyll contained in normally functioning plant cells. Ryther and Yentsch (1957) imply that this can be achieved by measuring chlorophyll only at the chlorophyll *a* absorption peak with a narrow-waveband light source. This procedure may reduce errors from the inclusion of the pheo-pigments (see Section II.C) to be expected in phytoplankton detritus, but this has not been substantiated by Odum *et al.* (1959). In view of the separately located enzyme systems involved in the production of assimilatory power and in later stages of cell growth and metabolism one could envisage occasions when the chloroplasts in damaged cells remained intact although carbon fixation was greatly arrested. There is no obvious way of overcoming this difficulty and it may well be that such errors account for a sizable fraction of the variability shown in Table VIII.

Another fault in the technique of productivity measurement under discussion lies in the assumption that chlorophyll *a* is the only pigment to be considered. Although chlorophyll *a* is the essential pigment for photosynthesis and must be given pride of place, the carotenes and xanthophylls (and chlorophyll *c* in much of the marine phytoplankton) cannot be ignored. It is now well established that these "accessory pigments" transfer light energy to chlorophyll *a*, enabling the utilization of wavelengths that would otherwise be ineffective. For a given intensity of daylight, therefore, the amount of carotenoid or other pigments would be expected to have some importance, even though, in the last analysis, the quantity of chlorophyll *a* may dominate the productivity relationship. Probably a simple linear function with chlorophyll *a* as dependent variable ought to be modified by a term containing the amount of other pigments. This modification would be expected to apply more to algae in the Chrysophyta and Pyrrophyta than to plants in the Chlorophyta, as in the latter chlorophyll *a* is generally the predominant pigment. Currie (1957) produced evidence of the importance of accessory pigments when he obtained a better relationship between $^{14}CO_2$ productivity data (probably a substantial fraction of gross photosynthesis) and *the sum total of all pigments* rather than between $^{14}CO_2$ uptake data and chlorophyll *a* values alone. Haskin (1941) has suggested that the loss of photosynthetic activity per unit chlorophyll noted in aging cells may well arise from a reduction in the amount of carotene present in such cells.

One further point of interest is worthy of mention whilst discussing the relation of pigments to the rate of photosynthesis. Yentsch and other workers at the Woods Hole Oceanographic Institution (see Ketchum, 1957) report that a linear relationship holds between the ratio of net to gross photosynthesis and the ratio of chlorophyll *a* to the total carotenoids in a water sample. Net photosynthesis was taken as the $^{14}CO_2$ uptake rate and gross as the figure obtained by L and DB experiments. The ratio net/gross varied from some 0.9 to 0.95 in healthy vigorously growing cells to 0.2 or less in excessively chlorotic plants and was undoubtedly an arbitrary but useful index of the general metabolic activity of a cell, showing the tendency of a plant to photosynthesize material of "permanent" use to the cell. However, the reason why a linear relationship of the form:

$$\frac{\text{net}}{\text{gross}} = K_1 \times \frac{[\text{ chlorophyll } a\ ]}{[\text{ carotenoids }]} + K_2$$

where K_1 and K_2 are constants, should hold is not all obvious. The linearity was only marked in work undertaken with pure cultures and applied to only a limited range of pigment ratios. Data obtained in offshore waters were less satisfactory and inshore data had little if any consistency, possibly due to the presence of detrital pigments.

H. OTHER METHODS

There are only a few remaining techniques of primary productivity measurement that do not fall into the categories already discussed.

The most important of these is designed to measure productivity, *in situ*, over large areas of ocean, by noting changes in the depth profiles of oxygen or phosphorus concentrations. These changes are measured in an entire column of water during several closely spaced cruises, or attempts are made to calculate nutrient replacement by eddy diffusion. G. A. Riley and J. H. Steel have taken a prominent part in the modern developments of this approach and a detailed discussion will be given in the last section of this review (see Section V.C).

There remain only methods based on the uptake of elements other than carbon, almost exclusively the uptake of phosphorus. Nitrogen would be as satisfactory an element to study were it not for greater analytical difficulties.

A study of the uptake of nitrogen or phosphorus has the disadvantage that the composition of phytoplankton with respect to these elements is so variable. Thus uncertainties are introduced into productivity measurements from the start. There are also analytical considerations.

For example, work in the Nanaimo laboratories of the Fisheries Research Board of Canada has shown that the smallest change in the *total* soluble phosphorus content of sea water that can be detected with statistical significance at the 0.05 level is about 0.15 μg-at P/l. Replicate determinations could, of course, improve matters but this involves more time and effort on what should be, essentially, a rapid technique. The exact detection limit depends somewhat on the level of phosphorus concentration in the sea water but if we assume that a figure of 0.1 to 0.15 μg-at P/l applies to sea water containing between about 1 and 3 μg-at P/l of soluble phosphorus then the minimum detectable change in phytoplankton crop that can be established by this method works out to some 120 to 180 mg C/m^3. The method is thus scarcely as sensitive as the carbon dioxide consumption measurement described in Section IV.D, although it is possibly rather less exacting to carry out than high precision pH measurements.

A point not sufficiently stressed in the literature is the necessity of measuring changes in the *total* soluble phosphorus (phosphorus in the filtrate through a membrane filter) rather than changes in the inorganic phosphorus content of samples. Sea water, especially from coastal areas, may often have at least as much soluble organically combined phosphorus present in the euphotic zone as there is inorganic phosphate (ref. e.g. Redfield *et al.*, 1937; Armstrong and Harvey, 1950; Pratt, 1950; Ketchum *et al.*, 1955; Pomeroy *et al.*, 1956; Collier, 1958) and a sizable fraction of this organic phosphorus *may* be available to growing plants, either directly or via rapid bacteriological degradation (see Harvey, 1940; Chu, 1946; Harvey, 1953c). Thus changes in the inorganic fraction alone can be misleading. A few authors have reported measurements of phosphate change, generally along with oxygen in L and DB experiments, which can be used to give estimates of productivity (cf. Riley, 1939b; Edmondson and Edmondson, 1947; Smayda, 1957) but the method has not achieved general acceptance.

It might be thought that the use of the radioactive isotope would overcome many of the limitations of phosphorus uptake measurements and lead to a very sensitive method, in much the same way as the use of the ^{14}C isotope has proved so fruitful. Unfortunately, this is not the case. The exchange of one carbon isotope with another was sufficiently slow to be ignored or could be accounted for adequately by a dark blank determination. Much of the phosphate entering an algal cell, however, appears to be extremely labile (ref. e.g. Gest and Kamen, 1948; Goldberg *et al.*, 1951; Rice, 1953) and is rapidly exchanged with cellular phosphorus, probably by means of a sequence of rapidly adjusting equilibria, so that there can be a rapid uptake of ^{32}P from solution without the intra- or extra-cellular phosphorus concentration decreasing to a significant extent.

This exchange behaviour is described in an excellent paper by Rigler (1956). The exchange rate is proportional to the concentration of living cells and inversely proportional to the concentration of inorganic phosphate, as each cell exchanges a definite fraction of its phosphate with the external medium at approximately a constant rate. Thus exchange is at a minimum with a small phytoplankton population present in water containing a high phosphorus content but one would estimate from Rigler's experiments in a freshwater lake that the exchange effect under most natural marine conditions would still be too great for the radiophosphorus isotope to be used with much success.

I. SOME RESULTS OF RATE DETERMINATION MEASUREMENTS

The total photosynthetic production of organic matter in the oceans as a whole has been the subject of much inspired guesswork. It was placed in the range of 4.4 to 20.8 × 10^{10} tons carbon per annum by Riley (1944), based on a mean estimate of 310 ± 180 g C/m^2/year. The estimate by Nielsen (1952b) of some 1.5 × 10^{10} tons of carbon a year is little different from the lower estimate given by Riley and both authors do no more than guess at the primary productivity in the Arctic and Antarctic, which may be a very significant fraction of the whole. It seems safe to say that the total oceanic photosynthesis is at least comparable with the terrestrial production of ca. 2 × 10^{10} tons a year (to use the much-quoted figure of Schroeder) and may even be several times greater (Ryther, 1959), especially if it is capable of supporting a potential global fish catch of 5 × 10^7 tons or more (Reay, 1954).

The average annual production beneath unit area is more uniform than might be at first supposed, the relatively low fertility of many tropical seas being offset by high mean radiation and a great depth of euphotic layer. Some 50 to 100 g C/m^2/year is now established as a reasonable range for subarctic waters. The annual crop in the tropics may not be much less than a half of this.

Local short-term production rates, however, can vary greatly. Algal cultures (*Chlorella*) under ideal conditions are capable of synthesizing at a rate around 5 to 6 g C/m^2/day (see e.g. Burlew, 1953; Thacker and Babcock, 1957, and refs. cited). The highest primary productivity value reported for the sea by modern methods approaches this (3.8 g C at Walvis Bay, Southwest Africa, reported by Nielsen, 1954), but such conditions are exceptional and, generally speaking, 0.5 g C/m^2/day may be considered to be a high rate of marine primary production. It seems likely (ref. especially Berge, 1957) that the production per unit area of sea surface is relatively constant over quite wide areas and charts of iso-production lines for a given ocean may eventually be common place.

The production of combined carbon per unit volume is more variable, as it depends upon both the light intensity and the crop density at any point in the euphotic zone (ref. Section V.C.2). Oliver (1957) has pointed out the difficulty of reconciling the rapid attainment of high oxygen supersaturation values, so often noted in the surface waters of inlets in still weather, with the order of primary production measured by ^{14}C uptake in the same general area. He has suggested (private communication) that this apparent discrepancy arises in shallow waters from the benthic production of oxygen from epiphytic or periphytic diatoms, etc., greatly exceeding the planktonic production. This must be guarded against when assessing the total productivity of any inshore area.

It is not the purpose of the present review to discuss the reasons for varying primary productivity. The basic causes are now well established (ref. e.g. articles by Gran, 1931; Harvey *et al.*, 1935; Nielsen, 1937b, 1952a, 1954, 1955a

TABLE IX. Selected examples of productivity measurements.

Location	Depth of sample	Time of year	Primary productivity			Method	Reference	Remarks
			mg C/m^3/hr	mg C/m^3/day ($_{24}$)	mg C/m^2/day ($_{24}$)			
English Channel near mouth	......	June	....	....	500	^{14}C	Nielsen, 1954	24-hr day. Between net and gross.
North Sea	Mean value for euphotic zone	April May	....	*ca.* 7 *ca.* 10	....	^{14}C	Cushing, 1957b, c	Between net and gross ....
North Sea	......	Annual range	....	....	200–1000	Phosphorus balance	Steel, 1956	See Section V
North Sea	......	Annual range	....	....	100–1500	^{14}C	Steel, 1957	Between net and gross
North Sea near E coast of England	......	May	....	....	220	^{14}C	Cushing, 1957c	Mean production. Between net and gross
North Sea near E coast of England	......	October February	....	....	110 5	^{14}C	Wimpenny, 1957	......
Kattegat (Baltic)	......	October	....	....	250	L and DB	Nielsen, 1937	Gross. About maximum production in a low production region
Oslo Fjord	1 m	March	....	*ca.* 100	....	Oxygen evolution	Gaarder and Gran, 1927.	Experiment lasted nearly a week. Probably a low estimate of net. Of historical interest
Danish coastal waters	......	Aug. max. March bloom (Dec. min.)	....	....	700 300 *ca.* 10	^{14}C	Nielsen, 1957c	Between net and gross
Western Barents Sea Arctic water in Bear Island current	Surface, max. 20 m	May	2 9	*ca.* 15 *ca.* 75	1300	^{14}C	Corlett, 1957	Between net and gross

TABLE IX. (Continued)

Location	Depth of sample	Time of year	Primary productivity			Method	Reference	Remarks
			mg C/m³/hr	mg C/m³/day (24)	mg C/m²/day (24)			
Western Barents Sea. Atlantic water south of Bear Island	Surface 50 m	May	0.4 0.7	....	275	^{14}C	Corlett, 1957	Between net and gross
Mediterranean Sea. 2 miles off French coast	5 m	Midsummer	....	1.5	30–40	^{14}C	Brouardel and Rinck, 1956	Between net and gross. Very similar in July and October
Eastern Atlantic 15 miles off Oporto	......	September	....	....	1000	^{14}C	Currie, 1957	Between net and gross
Eastern Atlantic 200 miles off Oporto	......	September	....	....	150	^{14}C	Currie, 1957	Between net and gross
Long Island Sound, east U.S.A.	1 m	Range for year (max. in Mar., July, Sept.)	....	15–350	....	L and DB	Riley, 1941a	Gross. Experiments lasting 2 to 4 days
Long Island Sound, east U.S.A.	......	Range for year	....	20–300	....	L and DB	Riley *et al.*, 1956 (Conover)	Gross
New England coastal waters	Surface	August	....	250	....	L and DB	Riley and Gorgy, 1948	Gross. Incubated on deck
Western Atlantic, New England slope water	Surface	August	....	75	....	L and DB	Riley and Gorgy, 1948	Gross. Incubated on deck
Western Atlantic off New York. Inshore Offshore	......	April April	....	....	850 1100	Chlorophyll and light	Ryther and Yentsch, 1958	Assumed to be gross
Western Atlantic. New England slope water	Surface	Summer	....	100	....	L and DB	Riley, 1939b	Gross. 3-day incubation open to criticisms outlined in text

Table IX. (Continued)

Location	Depth of sample	Time of year	Primary productivity			Method	Reference	Remarks
			mg C/m^3/hr	mg C/m^3/day ($_{24}$)	mg C/m^2/day ($_{24}$)			
Off New England coast on Georges Bank	Top 30 m	March	....	230	....	......	....	Gross
		April	....	....	950	L and DB	Riley, 1941b	Net
	Av. for euphotic zone	April	....	450	....	......	....	Gross
Gulf Stream near eastern U.S.A.	Surface	Summer	....	30	....	L and DB	Riley, 1939b	Gross. 3-day incubation open to criticisms outlined in text
Northeastern U.S.A. Narragansett Bay	Surface	Mean value for June to Feb.	....	250	....	L and DB	Smayda, 1956	Gross
Sargasso Sea Atlantic Ocean	......	Annual average	....	....	150	Estimated by various biological and physical reasonings	Riley, 1957	Approximately net. See Section V
Sargasso Sea Atlantic Ocean	Surface	Summer	....	20	....	L and DB	Riley, 1939b	Gross. Average for many areas. 3-day incubation. Open to criticisms outlined in text
Sargasso Sea Atlantic Ocean	Surface	May	....	0.5	....	^{14}C	Nielsen, 1954	Between net and gross
		May	....	....	40–50			
East Gulf of Mexico	Surface	Av. value for euphotic zone	....	*ca.* 50	....	L and DB	Riley, 1938a	Low production area like Sargasso. One week incubation time and hence results open to criticisms outlined in text
Puget Sound. NE Pacific coastal waters	1 m	August	45	*ca.* 300	....	L and DB	Gran and Thompson, 1930	High crop density area. Gross
Equatorial Pacific productive area near coast of Ecuador	Surface	Autumn	....	15	500–1000	^{14}C	Holmes *et al.*, 1957	Between net and gross

TABLE IX. (Concluded)

Location	Depth of sample	Time of year	Primary productivity			Method	Reference	Remarks
			mg C/m^3/hr	mg C/m^3/day ($_{24}$)	mg C/m^2/day ($_{24}$)			
Equatorial Pacific 11° N, 115° W	Surface	Autumn	....	0.15	10	^{14}C	Holmes *et al.*, 1957	Between net and gross
Equatorial Pacific east of 160° W	20 m	November	max. *ca.* 1.5 av. *ca.* 0.4 min. *ca.* 0.05	*ca.* 15 *ca.* 5 *ca.* 0.5	....	^{14}C	King *et al.*, 1957; Doty, 1956, 1957	Between net and gross. Incubated at about 1500 ft-candles; *in situ* production probably less
Equatorial Pacific between about 30° N and 30° S	Surface	March March	....	.... 1.5–5	100–250	^{14}C	Nielsen, 1954	Between net and gross
Pacific Ocean Hawaiian Chain. 1 mile from shore 15 miles from shore	At or near surface	....	4 0.1	*ca.* 40 *ca.* 1	....	^{14}C	Doty and Oguri, 1956	Between net and gross. Data to illustrate "Island mass effect" (see Section V)
Pacific Ocean Hawaiian Chain. 4 miles from shore 15 miles from shore	Surface	August	0.5 0.1	*ca.* 5 *ca.* 1	....	^{14}C	Doty, 1955	Between net and gross. Incubated at about optimum light. *In situ* production probably less
Sea of Japan (Middle Part)	Surface	May	....	120	2000	^{14}C	Sorokin and Coblenz-Mishke, 1958	Between net and gross. Just after spring bloom
North Pacific productive area near end of Aleutian chain	Surface	November	....	70	....	^{14}C	Holmes, 1957	Between net and gross
North Pacific Aleutian Ridge	......	Summer	....	50	....	Phosphorus consumption	Fukai, 1954	Very approximate, net
Off coast of Japan	Surface	August	10–20	*ca.* 75–100	....	^{14}C	Miyake *et al.*, 1954	Between net and gross
South Atlantic inshore by SW coast of Africa	Surface	December December	....	 0–6000	500–4000	^{14}C	Nielsen, 1954	Between net and gross. Most productive area found on Galathea cruise
Arctic Ocean Ice Island T-3	......	July (mat)	....	....	24	^{14}C	Apollonio, 1958	Between net and gross. Evidence population light-starved

and 1957c; Iselin, 1939; Sverdrup and Allen, 1939; Doty, 1955 and 1956; Harvey, 1957b; amongst many others). Radiant energy is a dominant factor in early spring and late autumn in arctic and subarctic regions and for waters beneath the arctic ice cap (Apollonio, 1958), but for much of the summer, and for all the year in the tropics, the light pattern would not be expected to exert a major influence. The replenishment of nutrients into the euphotic zone through a stabilized thermocline or halocline will govern plant growth in most circumstances. This replenishment can be brought about by upwelling and by tide or wind mixing but must not involve too violent a process if the crop is not to be dissipated more rapidly than it is formed. Where "old" water persists due to excessive stability, or in very shallow inshore coastal waters, the crop growth will depend greatly on the rate at which nutrients are remineralized from plant matter by the agency of bacteria, zooplankton or, possibly, free enzymes. The availability of essential organic or inorganic "growth factors", in addition to nitrogen and phosphorus, can conceivably exercise a dominant role, especially near land, and can be of the greatest importance in determining the "succession of species".

The pattern of primary production generally resembles the behaviour already outlined for standing crop density in the last Section, with several "outbursts" throughout the year in temperate regions. The magnitudes to be expected are illustrated in Table IX where, as with the crop values given in Table VII, the present author has selected a limited number of results from publications of American and European origin. The factors suggested in Section I.B. have been used, as appropriate, with a PQ value of 1.2 being assumed for L and DB experiments. Results have been rounded off freely as they are only intended to illustrate magnitudes and in most cases a greater precision of reporting is scarcely justified.

Where only values for mg $C/m^3/hr$ are available a *rough* estimate of the production as mg $C/m^3/day$ has been attempted to give the maximum number of directly comparable data. Little can be done to convert values from mg $C/m^2/day_{(24)}$ to mg $C/m^3/day_{(24)}$ when the latter data are not reported in the original publication. The conversion of surface production in mg $C/m^3/day_{(24)}$ to values for mg $C/m^2/day_{(24)}$ in the whole euphotic zone is also not feasible unless details are reported for water transparency and the distribution of crop within the euphotic zone. In the summer at subarctic stations a factor between about 5 and 20 would probably apply for this last relationship. In the tropics the factor is likely to be nearer to 100.

SECTION V. PRIMARY PRODUCTIVITY

We have deferred until this final section any detailed discussion of the measurement of primary productivity in nature and of methods designed to predict *in situ* productivity with the minimum amount of experimentation.

It appears opportune, however, first to give some brief consideration to the general growth kinetics of unicellular organisms, in particular the photosynthetic unicellular algae that comprise the marine phytoplankton. Before discussing kinetics we will introduce the concept of the ***Productivity Index*** (PI) of a water mass. This concept has a potential usefulness which, in the opinion of the present writer, has been insufficiently appreciated by most workers.

A. PRODUCTIVITY INDEX

A knowledge of either the standing crop or the short-term net primary productivity of a phytoplankton population gives little information as to the inherent "production intensity" or "vitality" of the population. A large crop may be photosynthesizing at a comparatively low rate or, alternatively, a high rate of production can arise from a small crop with vigorous growth characteristics (see e.g. Verduin, 1956a). It is clearly desirable, therefore, to know both crop density and net primary productivity if we are to decide on the relative fertility of water masses and their endemic populations. Despite this, nearly all investigations still report only one or the other property and the reader is left in doubt as to the true reason for a given rate of primary productivity.

The influence of light is also important as the production per unit crop will be roughly proportional to the light intensity until the optimum illumination of some 0.1 to 0.15 ly/min is reached.

An accurate assessment of the fertility of a water sample therefore requires a knowledge of the rate of net photosynthesis per unit crop of plant material at a known sub-optimal light value. Rates and crop should be measured on a combined carbon basis, considering the carbon per unit volume rather than the amount beneath unit area, as in the latter case the light parameter is too complex to be used directly. Such a measure has been defined in Section I.A of this review, with 1 ly/min of photosynthetically active light taken as the illumination unit, and will be referred to as the productivity index (PI) of a water sample. If the crop is in a state of exponential growth the PI is a measure of the rate constant at unit illumination as

$$\mathrm{PI} = \frac{\text{Rate of production of crop (at unit light intensity)}}{\text{Amount of standing crop}}$$

$$= \frac{\mathrm{d}p}{\mathrm{d}t} \times \frac{1}{p} = k, \text{ at unit light intensity}$$

where p is the crop, t is time and k a rate constant (see later in this section).

The main practical difficulties in measuring mutually comparable PI values are to know the relevant light intensity factors and the amount of carbon associated with the plant cells. In nature the latter may be an insoluble problem. Ideally, rates should be measured at sub-optimal light values in the linear portion of a P vs. I curve (see later). It may be that the PI at optimum light intensity is a more practical unit than measurements corrected to a hypothetical unit illumination, as advocated above, but the latter have the merit of giving a measure of the relative response of phytoplankters to low light intensities, which is a sensitive function of their physiological condition and immediate past history.

For much of the year the PI will measure the nutrient value of a water sample but this nutritional factor cannot be completely separated from effects that arise from the metabolic condition of the algae contained in the water, a condition which will largely reflect the immediate prehistory of the phytoplankton crop. Senescent cells or endospores may have poor growth characteristics even in fertile waters, until sufficient time has elapsed for a rejuvenation or reactivation process to take over and, conversely healthy well-nourished cells can continue vigorous growth for several divisions even in the most barren waters. The latter possibility, however, is unlikely in a natural population, although easily demonstrated in cultures.

There are, unfortunately, very few values for a PI available in the literature. Several authors have appreciated the desirability of reporting photosynthetic rates on a unit crop basis and a few have recorded, simultaneously, precise illumination data but in no instance is the productivity index, as defined in this review, used and in only two or three cases can the PI be calculated with any precision.

As mentioned in Section IV.G there is evidence that gross primary productivity (which may often approach the net value) can be related to the chlorophyll content of the water and to the illumination at sub-optimum light intensities by the approximate equation given in Section I.B. It follows that the productivity index (measured using gross productivity) should have a value not far removed from unity, say 0.5 to 2 $(\text{hours})^{-1}(\text{ly/min})^{-1}$.

The present author has only been able to find two papers where a natural *marine* PI can be calculated with precision using data reported directly in terms of combined carbon. In the classical paper by Jenkins (1937) the PI evaluates to around 2 for *Coscinodiscus excentricus*, and from the data given by Ryther and Vaccaro (1954), one calculates a value near to 0.8 for *Nitzschia closterium* in culture. In several papers the PI can be calculated if a conversion from chlorophyll to standing crop carbon is assumed but this assumption greatly reduces the precision of any estimate. A value of about 2 can be derived from the data of Shimada (1958). Results given by Holmes (1957) and Holmes *et al.* (1957) lend to PI values that centre around about 0.5 and 2.0, but with many values outside this range. Similar conclusions are reached by studying the data of King *et al.* (1957). McQuate (1956) reports directly in carbon units and although the work concerns a fresh water lake it is interesting to find the PI value again near to unity. Miyake *et al.* (1954) also report data expressed on a carbon basis, this time for Japanese coastal waters, but the light intensity values are uncertain and the PI comes out extremely low, about 0.02, indicating an overestimate of the phytoplankton standing crop due to the inclusion of zooplankton and detritus.

It is unfortunately not possible to calculate precise PI values from the data collected by Riley *et al.* (1949) in their table VI. The light intensities are given but standing crop carbon has been derived from chlorohpyll or HPPU measurements and the time unit is a day rather than an hour. One could hazard that, as a very rough approximation, the PI is around 0.2 on Georges Bank, 0.4 in Long Island Sound and 0.5 in the Sargasso and Dry Tortugas but very little reliability can be assumed for these estimates (see also Riley, 1946).

The values given by Currie (1957) have the standing crop expressed in pigment units and the light intensity values are difficult to estimate. Nevertheless the PI (which is probably near to 0.5) can be seen to be fairly constant with depth in the euphotic zone and is rather greater in oceanic areas than in coastal waters. This surprising fact emerges despite the fact that the primary productivity was found to be over twenty times as great near the coast than in the open Atlantic Ocean some 200 miles or more from land, where the PI was as high or higher. R. W. Holmes (private communication) confirms this type of behaviour in the eastern sub-tropical Pacific.

Steel (1957a) has calculated data similar to PI values except that the light intensity parameters are purely relative. Only relative PI values are thus obtainable but these are seen to be approximately constant with depth in the euphotic zone, although perhaps a little lower at the surface than elsewhere. R. W. Holmes (private communication) finds a decrease of PI in the detrital layer formed at 50 to 100 m in the sub-tropical Pacific Ocean.

The data given by Cushing (1957b, c) are recorded in terms of the fractional increase of crop carbon per day (20 to 80%, with a mean of about 50% in the North Sea) but the light intensity term is missing and no accurate PI value can be obtained. Berge (1957) takes into account the effect of light on primary productivity by evaluating productivity as mg C/l/hour/lux, termed by him the "production capacity" of the water. He gives interesting charts to show isolines of "production capacity" ranging from about 30 to 300 mg C/m^3/hour/ly/min. However, no crop was measured, which prevents the PI being calculated. As the crop is probably in the range of 30 to 300 mg C/m^3 in the Norwegian Sea, the PI values may again be near to unity.

Thus it seems likely that the productivity index will be found to lie in the range of 0.1 to 5 $(\text{hour})^{-1}(\text{ly/min})^{-1}$ for most waters but as yet an insufficient number of precise values have been obtained for one to draw many conclusions as to the effect of location, nutrients, time of year, etc., on the index. The PI appears to be fairly constant with depth at any given time and may not necessarily be any less in oceanic areas than nearer inshore, but its determination will probably have to be carried out with considerable precision if the more subtle patterns of relative fertility are to be resolved.

The great practical value of the index, apart from indicating the relative fertility of sea areas, may be to lessen the amount of experimentation necessary for measuring primary productivity. If it can be established that the PI of a given area is fairly constant with depth and location and can be found by a limited number of determinations then it will only be necessary to carry out standing crop measurements in order to evaluate the primary productivity. Standing crop estimations will almost certainly always be more rapid and convenient to undertake than the measurements of photosynthetic rates and sampling problems are considerably reduced. Large volumes of water can be taken (ref. Section III.A) and the algal cells do not have to be treated with such extreme care.

Before concluding it is worth mentioning that the ratio of net to gross photosynthesis (see Ketchum, 1957, reporting on the work of Yentsch and others) appears to be an effective index of the general metabolic activity of an algal crop, certainly in cultures. This "productivity" index cannot be used to calculate photosynthetic rates from crop measurements but it may be of some value as a "fertility indicator" and there is evidence that the ratio reflects the nutrient depletion in stabilized summer surface waters.

B. GENERAL GROWTH KINETICS

It is obvious that light energy plays a dominant part in the growth of photosynthetic organisms and this aspect will be discussed in detail later in this section. However, the unicellular algae which comprise most of the marine phytoplankton have many growth characteristics in common with non-photosynthetic unicellular organisms, such as Protozoa, yeasts and bacteria (ref. e.g. Fogg, 1957b). The latter have been the subject of intensive investigations during which most of their growth patterns have been determined. The response of these cells to their environment can often be described mathematically from models assuming only the generalized interaction of enzyme systems restricted in space by cellular boundaries of somewhat critical dimensions. Such unifying and challenging concepts are expressed with admirable clarity by Hinshelwood (1946) in his treatises on the kinetics of the bacterial cell.

By comparison with the work on bacteria the number of kinetic studies on unicellular algae in culture are very limited (e.g. Pearsall and Loose, 1937; Ketchum, 1939b; Riley, 1943; Ketchum and Redfield, 1949; Ketchum *et al.*, 1949; Winokur, 1948a; Smayda, 1957; Kain and Fogg, 1958; and other references cited later) and many investigations have been directed to other ends with kinetics only of subsidiary interest. Certain factors emerge, however, which are of some importance to the measurement of primary productivity and which merit discussion in the present review.

1. KINETICS OF UNICELLULAR GROWTH AND DECAY

a. DORMANCY OF LIVING CELLS, THE "LAG" PHASE OF UNICELLULAR GROWTH

It is well known that on inoculating a fresh medium with a sparse population of bacteria cells, a variable and often lengthy "lag" interval may occur before a vigorous exponential growth of the bacteria is re-established. A similar behaviour is found with unicellular algae in culture (ref. e.g. Ketchum *et al.*, 1949; Fogg, 1953; Kain and Fogg, 1958) although a distinction must be made between an increase in the number of cells and an increase of cellular substance; the latter is often subject to less lag than the former (cf. Harvey, 1953a). The analogous phenomenon in nature is found in the delayed blooming of spring phytoplankton crops which, although influenced by lack of radiant energy and by certain hydrodynamic factors, probably depends also on a true growth lag (see Fogg, 1957b). Similarly, throughout the summer, the bloom of successive algal species is delayed by factors which may not always be physical or, in the commonly accepted sense, nutritional.

The growth of a cell and the reproduction of its matter is connected with the enzymes responsible for the catalysis of critical growth stages and these enzymes are themselves intimately involved with their own workings, interactions and synthesis. It can be shown that the mathematical consequence of all this is that the ratios of various kinds of enzymes in an organism will eventually take up stable relationships. It is to be expected that the proportions of the constituents that are finally established will lead to a maximum rate of growth in any particular environment.

If two or more substances, $x, y, \ldots.$ are formed enzymatically and the rate of formation of one substance depends on the concentration of another, e.g., in its most simple form:

$$\frac{dx}{dt} = A.y, \ \frac{dy}{dt} = B.x, \ \ldots\ldots \text{ etc.}$$

then it follows mathematically (ref. e.g. Hinshelwood, 1952, 1953a, b) that the eventual production of each substance with time will occur exponentially. For some time before this exponential stage is reached the amount of each component may fluctuate, however, although the fluctuations will be small when compared with the subsequent exponential increase.

Thus a lag phase is to be expected in any system of enzymatic reactions, such as occur in cellular synthesis, when the system is thrown off balance by the temporary removal of a critical reactant. The precise cause of the lack of balance during the lag period of phytoplankton or bacterial growth is most likely to be the shortage of a critical growth promotor in the medium, a promotor produced by the organism itself (ref. e.g. McVeigh and Brown, 1954; Harvey, 1947; Kain and Fogg, 1958) or present in the medium to which the organism was initially adjusted. The shortage is brought about by dilution, either by the experimenter during culture transfer or, in nature, by the intrusion of foreign water masses or the dilution effect of winter mixing during a period of low photosynthetic activity.

Such a hypothesis can explain many of the lag phenomena found in bacteriology (effect of cell age, cell number, etc.) and is capable of experimental verification in certain cases (ref. Hinshelwood, 1946), but it is certainly not the sole explanation of all observed growth dormancy.

There are other possibilities and contributing factors. Thus it is possible that a very small proportion of the cells present during a growth induction period may have an activity far above the average and multiply in an exponential manner whilst the other cells are largely static. The lag phase is then at an end when the number of these active cells becomes a significant fraction of the total population. Riley (1943), in experiments with a marine *Nitzschia*, found very erratic behaviour in the lag stage which was not clearly related to the nutritional adequacy of the medium. During the lag phase respiration was comparatively high and a large fraction of the cells were in a senescent state. However, photosynthesis on a unit crop basis appeared to be quite marked and Riley inferred that the lag period might be associated with a vigorous growth of some of the population balance by a correspondingly high death rate of others.

b. CELL DIVISION AND THE LOGARITHMIC PHASE OF GROWTH

Cell division in a growing organism almost certainly comes about, in the last analysis, because of a "scale effect", that is, because of the disproportionate reduction of surface area to volume in a growing cell. It is only through a surface that the cell can gain or lose metabolites and the most obvious implication is that division occurs when the concentration of some key substance in the cell eventually reaches a critical concentration, owing to the retarded ability of the cell to lose the substance by diffusion. A mathematical model based on this premise will predict, qualitatively, most of the observed behaviour of cell division in bacteria, including the effect of chemical and physical factors known to speed up or slow down cell division (ref. Hinshelwood, 1946, 1952).

Even in pure cultures the dimensions of any cell at the time of division and the rate of division of an individual algal cell can vary greatly about a mean.

The *Bacillariophyceae* reproduce by division parallel to valve faces, one daughter being smaller in linear dimensions than the other by double the thickness of the connecting band. A continual decrease in mean cell size with time is thus found in nearly all diatoms, although lightly silicified species appear to have some adjustment. This reduction may persist for many months, resulting in a linear decrease of 30 to 40% in cell dimensions, giving rise to cells having only 25% or less of the original volume.

This decrease can be used to estimate the number of generations of diatoms produced, in nature, in a given time (ref. e.g. Cushing, 1953). The process is finally arrested either by death or by auxospore formation.

The Dinophyceae also reproduce by cell division, generally obliquely along the longitudinal axis, but there does not appear to be any great permanent variation in size although one half of the organism will be smaller than the other for some time. Similarly the motile unicellular species of Chrysophyceae found in sea water (in particular the Coccolithophoridae) show cell division. The Myxophyceae are more varied in their modes of reproduction, using either cell division, vegetative budding, or spore formation. The marine planktonic xanthophyceae (eg. the *Halosphaera* and *Meringosphaera*) breed by the asexual process of zoospore swarming. This last process results in wide size variations of a temporary nature but no information is readily available as to the normal variations to be expected in adult cells.

The Chlorophyceae have a great diversity in their breeding habits. Most marine planktonic forms, such as *Chlamydomaonas* and *Carteria*, probably propagated by longitudinal cell division (ref. e.g. Fritsch, 1935). There is certainly a great variation in the size of individual cells in freshwater planktonic Chlorophyceae, but the more mature the culture the less the size variation of individuals (ref. e.g. Ketchum and Redfield, 1949; Ketchum *et al.*, 1949). In a species of *Chlorella*, daughter cells were produced that, for an appreciable period, had markedly different growth properties from the parents (Tamyia *et al.*, 1953; Sorokin, 1957; Sorokin and Myers, 1957).

The variability of the generation time of individual bacteria cells (the time between the formation of a cell and its division into daughters) is generally very great and unicellular algae behave in much the same manner. The variation is not strictly Gaussian in its distribution and there is a greater frequency of large deviations than probability would predict. Nevertheless, the general behaviour suggests some mean value for a division time which is advanced or retarded in each individual cell by a multiplicity of random factors.

Whatever the exact form of the reproductive process the total matter and, statistically, the total number of cells will eventually increase in an exponential manner. (This, of course, presupposes that sufficient conditioning has occurred to overcome any lag.) The increase of total cell substance or cell numbers then follows an equation of the form:

$$\frac{dn}{dt} = k.n \qquad \ldots\ldots 4$$

$$\frac{dp}{dt} = k.p$$

where n or p are the number of cells or the amount of cellular substance, respectively, and k is a constant depending on the organism and its environment. If k is sensibly constant over a time interval, t:

$$\ln.n_t = \ln.n_0 + kt$$

or:

$$\ln.p_t = \ln.p_0 + kt \qquad \ldots\ldots 5$$

where n_0 and n_t, p_0 and p_t are the corresponding values at zero and time t, respectively. k has the dimensions T^{-1} and probably is best expressed in $(\text{hours})^{-1}$ for phytoplankton work. If t_g is the *mean* generation time for a cell (in hours):

$$t_g = \frac{0.7}{k} \quad \text{or:} \quad k = \frac{0.7}{t_g} \qquad \ldots\ldots 6$$

"Logarithmic" growth is only sustained in experimental or natural populations for a limited time, owing to the intervention of factors to be discussed later, but it is often obeyed with precision for short periods, even if the concurrent mortality of cells is appreciable. Should the mortality of barren daughter cells be as great as 10% of births, departures from an exponential equation are not serious. Any marked changes of mean cell size in cultures generally occur either early or late in the exponential growth period and so may have little effect on the form of population increase. However, the increase of cell substance (dry weight) is often more regular than the increase of cell number and shows much less variation from species to species (ref. e.g. Winokur, 1948a; Ketchum *et al.*, 1949). The increase of individual molecular components in the cells will be much less regular as the composition of cells changes with time, due partly to changes in the external medium (ref. e.g. Pearsall and Loose, 1937; Fogg, 1953).

"k" will depend on the environment of the algal cells and the class and genus of the algae. There are even very marked differences amongst the species of a given genus (cf. Winokur, 1948a, who studied 8 species of *Chlorella*). It is remarkable, therefore, how nearly constant k values are for marine phytoplankters (mainly diatoms) as reported by various workers. Although data have generally been reported as generation times for some indefinite condition of light and temperature these are generally near the optimum and the results are rarely outside the range 0.02 to 0.15 $(\text{hours})^{-1}$. For example Braarud (1944) reported about 0.15 for small diatoms decreasing to 0.07 to 0.05 for larger species, the values given by Smayda (1957) are near to 0.05 and Harvey (1934b), quoting Gran for a marine *Chaetoceras*, suggested a value of about 0.12. For *Phaeodactylum tricornutum* k is approximately 0.06 (Ketchum, 1939b; Raymont and Adams, 1958) and for a marine *Nitzschia*, Riley (1943) gives about 0.02 for growth under conditions of rather poor illumination and nutrition (ref. similar value for a marine *Astericnella* by Kain and Fogg, 1958). The generation time found by Ragotskie and Pomeroy (1957) for a *Gymnodinium* was 12 hours, indicating a k also around 0.06 $(\text{hours})^{-1}$. The t_g value for culture of *Gonyaulax polyedra* is nearer 2 days (Sweeny and Hastings, 1958). McLeod (1957) gives

t_g as 12 hours for the chlorophyte *Dunaliella euchlora*. For the total phytoplankton population in the English Channel Harvey *et al.* (1935) estimated k as about 0.035 $(\text{hours})^{-1}$. Braarud (1937) found the k values under rather low illumination were very similar for a variety of diatoms.

However, under the diverse conditions found in the sea, variations in k are bound to be considerable, depending on species, light conditions, temperature, and the nutrient properties of the water. Certainly k values for warm tropical waters may be several times as great as the values given above for temperate and subarctic regions (cf. Wood, 1958). Also, it is difficult to decide how different the k values found for cultures under continuous illumination may be from those pertaining to natural populations of the same species that are subject to alternate periods of light and dark. It is clear from the work of Sweeny and Hastings (1958) that this can have an effect on the time of day during which cell division occurs but the effect on t_g may not be very great. Lund (1950) found that the division rate of *Asterionella* was roughly proportional to the daily total light intensity in a freshwater lake, rising to a maximum of about once a day at 3 ly/day. From these data Cushing (1959) has estimated that the average division time of diatoms in the euphotic zone of the central North Sea would also be about once a day for much of the year.

Exponential growth in nature will only occur, even as an approximation, during the "blooming" of phytoplankters, notably at the spring outburst in temperate seas. From the above it can be seen that a bloom (say a 1000- or 10,000-fold increase in cell number) should take under a week, some 100 to 150 hours, which is in accord with common observation. Also, if we assume that a mean illumination of some 0.05 to 0.1 ly/min was operative when the generation times quoted in the literature were determined, then a productivity index for temperate seas of near to unity is clearly of the correct magnitude. (The PI, it will be remembered, is defined as the k value, $\frac{dp}{dt} \times \frac{1}{p}$, at a hypothetical unit illumination of 1 ly/min).

c. Stationary phases at maximum population

The cessation of growth at the end of the logarithmic phase can be brought about by either one or the other of two factors, a depletion of essential nutrients or an inhibition of growth due to substances produced by the population itself (ref. e.g. the chemical aging and inhibiting factors described by Pratt, 1943; Levring, 1945; Lefevre *et al.*, 1951; Rice, 1954; Jorgenson, 1956; Grøntved and Nielsen, 1957; Kain and Fogg, 1958). Cells will continue to divide when nutrients are depleted (cf. Ketchum, 1939b; Riley, 1943; Harvey, 1953a), but this only delays the onset of the stationary phase and eventually growth must stop.

It is irrelevant to the present review to discuss the critical concentrations of nutrients below which growth ceases, except to remark on the regrettable paucity of such data for marine planktonic algae.

If exponential growth persists until a limiting nutrient is practically depleted then it is easy to show that the maximum population resulting from exponential growth must be proportional to the initial concentration of this nutrient. This relationship will not hold, however, if toxic products are inhibiting growth and a plot of maximum population against nutrient concentration is then no longer linear but falls off at high population levels. Both types of limitation can be demonstrated in cultures but we have no knowledge of their relative importance in the sea. The comparatively sparse population found in such a well-buffered medium as sea water tempts one to suppose that nutritional factors generally dominate the growth patterns of marine algae *in situ*.

If nutrient exhaustion is the limiting factor it seems reasonable to assume that the concentration c of a limiting nutrient falls at the same rate at which algal substance is formed, i.e.:

$$\frac{-\mathrm{d}c}{\mathrm{d}t} = k_2 . \frac{\mathrm{d}p}{\mathrm{d}t} \qquad \ldots\ldots 7$$

where k_2 is a constant for much of the growth phase. Over quite wide ranges, algal growth rates are known to be almost independent of the concentration of nutrients and we might expect, therefore, that the rate constant k' in the expression:

$$\frac{\mathrm{d}p}{\mathrm{d}t} = k'.p \qquad \ldots\ldots 8$$

is related to c by an equation of the same form as the Langmuir isotherm (see Hinshelwood, 1946), viz.:

$$k' = \frac{k.c}{k_3 + c} \qquad \ldots\ldots 9$$

where k_3 is a further constant and k is the constant previously defined as applying when the nutrient environment was fully adequate (ref. equations 4 and 5).

Combining equations 7, 8 and 9 gives an overall expression for nutrient controlled phytoplankton growth of the form:

$$k.t = 1 + \left[\frac{k_3}{c_0 + k_2.p_0}\right].\ln\left[\frac{p_t}{p_0}\right] - \left[\frac{k_3}{c_0 + k_2.p_0}\right].\ln\left[\frac{c_0 + k_2\,(p_0 - p_t)}{c_0}\right] \qquad \ldots\ldots 10$$

where c_0 is the concentration of a critical nutrient at $t = 0$.

k_3 has the dimensions of a concentration and mathematically expresses the limiting nutrient concentration about which so little is known in marine studies. Nevertheless k_3 may often be small with respect c_0 and over a considerable range may be neglected. For this time the last equation then reduces to equation 5, the expression given previously for unrestricted logarithmic growth.

As c_0 approaches k_3, k_2 may change and even were it still constant a simple exponential law would no longer be obeyed. Nutrient exhaustion is complete and the growth rate falls rapidly to zero.

The assumptions underlying equation 10 have been substantiated for unicellular reproduction with various organisms and there are good reasons to assume that the equation is operative for algal growths unaffected by self inhibition.

Attempts have been made to treat unicellular growth by the more commonly used population growth equations applied to macrobiological systems. In these equations reproductive and mortality coefficients are used which comprise "intrinsic" components and "population dependent" components, assumed to be related in a simple linear fashion:

$$\text{Reproductive coefficient} = a - b.p$$
$$\text{Mortality coefficient} = c + d.p$$

from whence one can deduce the Verhulst "logistic" equation for an isolated system:

$$\frac{dp}{dt} = k.p - m.p^2 \qquad \ldots\ldots 11$$

k is the difference between intrinsic factors $(a - c)$ and m is the sum of population dependent factors $(b + d)$.
Integration gives:

$$k.t = \ln p\left(\frac{k}{m} - p_0\right) - \ln p_0\left(\frac{k}{m} - p\right) \qquad \ldots\ldots 12$$

When either p_0 and m or when p alone are sufficiently small this equation reduces to the simple exponential form of equation 5.

Equation 12, although less complex than equation 10, has the disadvantage that it gives no quantitative account of the effect of varying nutrient concentrations. The significance of the constants b and d are not clear when speaking of unicellular organisms in liquid cultures and the logistic equation does not provide a convincing fit for some data on yeasts and algae (ref. e.g. Richards, 1928; Winokur, 1948a).

Turning now to the case of growth inhibited by self-produced toxins rather than nutrient deficiencies, we may relate the constant k' (equation 8) to k (equation 4) by a relation:

$$k' = k - \text{function}(x)$$

where x is the concentration of a growth inhibitor generated by the cells into the surrounding medium. We must assume this function linear if the subsequent kinetic problem is to be solved with any practicable simplicity. An equation:

$$k' = k - K_1.x \qquad \ldots\ldots 13$$

is therefore adopted and, although clearly an oversimplification, probably serves adequately for much of a growth cycle. K_1 is a constant.

It seems reasonable that x and p are related by the expression:

$$\frac{dx}{dt} = K_2 . \frac{dp}{dt}$$

where K_2 is a constant, for that part of a growth cycle where mortality is not very high, whence:

$$x = K_2(p - p_0) \qquad \ldots\ldots 14$$

if x is initially negligible.

Hence from 8 with 13 and 14:

$$\frac{dp}{dt} = (k + K_1K_2p_0).p - K_1K_2.p^2 \qquad \ldots\ldots 15$$

The formal resemblance of equation 15 to equation 11 is obvious and it leads to an analogue integration.

It is interesting to observe that in one treatment of the problem of self-inhibition an auto-intoxication factor has been incorporated into the logistic equation 11 by including a self-inhibiting term in addition to the mp^2 term (see e.g. Volterra, 1931). This leads to an integro-differential equation of great complexity which is assumed to look after growth kinetics for the *entire* growth cycle, viz.:

$$\frac{dp}{dt} = k.p - m.p^2 - s.p.A \qquad \ldots\ldots 16$$

where s is a coefficient of autotoxicity and A a parameter which is a function of p and t and which for simplicity must be written as:

$$A_{(t)} = \int_0^t p(\tau)d\tau$$

Even in this simplified form the solution is complicated (see e.g. Kostitzin, 1939) and does not, in practice, accurately represent the full growth and decay of a bacterial population in an undisturbed medium. It is not clear in unicellular growth studies exactly which mortality factors are expressed by m that are not of a self-inhibitory origin, but presumably this term looks after nutritional factors, toxins of very short life, and any parasitic diseases aggravated by a high population density.

In the opinion of the present writer any attempt to account for the full growth characteristics of a population *from start to finish* by *one* equation of general applicability, leads to little more than an exhibition of mathematical agility. For work in cultures a single curve may partake of logarithmic, linear, and autocatalytic sections (ref. e.g. Burlew, 1953). The use of such equations for various sections of the curve is undesirable, in as far as the equations have no obvious theoretical justification, but this practice has a short-term empirical value.

In order to sustain the maximum growth in algal cultures we determine the "optimum catch point" (where the PI is at a maximum) experimentally and the supporting medium must be slowly replenished to prevent the accumulation of toxins (ref. e.g. Ketchum *et al.*, 1949; Wassink, 1954; Thacker and Babcock, 1957).

In nature even less obedience to exact mathematical formulations can be hoped for as not only may phytoplankton populations be limited by both nutritional and auto-inhibitory factors but superimposed on these will be losses due to grazing and disturbing hydrodynamical influences (discussed later). These may well outweigh all other coniderations, except for rare occasions or when dealing with limited and partially controlled ecological systems, such as small shallow bays and lakes. For much of the summer, natural populations would be expected to be in a state equivalent to the later part of the exponential or the earlier section of the 'stationary' states as found with cultures.

A stationary state need not necessarily imply the absence of all metabolic activity. Ryther *et al.* (1958) have shown that a large natural diatom population, under nitrogen-starved conditions, may photosynthesize quite vigorously during the day but only to respire the lipid or carbohydrate thus formed during the following night, giving very little *net* growth.

d. Death, resting states and senescence

If adverse conditions are maintained for a sufficient length of time, unicellular organisms will eventually die. The decrease in the number of living cells with time is often approximately logarithmic, i.e.:

$$\ln n = \ln n_0 - k_4.t \qquad \ldots\ldots 17$$

where n_0 and n are viable cells at the commencement of a population decline and after a further time t_2."k"$_4$ is a death rate constant.

The implications of this equation are interesting as it is devised mathematically on the assumptions that the death of a cell is not influenced by the number of other survivors and that the chance of a cell dying in a short time interval is independent of its past history. We suppose, therefore, that during the decay phase of a unicellular growth cycle an unsatisfactory environment initiates a general decrease of all cell functions. Growth slows down and when the enzyme functions of a system are sufficiently disorganized from the peak of autosynthetic efficiency there occurs a random chance that a conjunction of several adverse events will expose the cell to fatal consequences, a state of affairs analogous to the radioactive decay of an atomic nucleus.

Cell death is not restricted to the post-logarithmic stage of a growth cycle (e.g. Riley, 1943; Cushing, 1955) but its effects are generally more apparent and significant at this stage and the frequency of occurrence may be expected to be greatest.

The death of a bacterial or algal colony will not continue until the complete extinction of the species, unless conditions are most unfavourable. A small but variable proportion of the population survives because of greater resistance. There may be a more or less changed structure in the surviving cells and they are said to be in a resting or spore form. The normal constituents of the cell are thus capable of rearrangement to give a state of greater stability, concurrent with lower activity, and this rearrangement is probably initiated by a critical concentration of promoter within the cell, as is the case with the stimulation of cell division.

Resting spore formation is not brought about by adverse environmental factors, as is often suggested, but rather the process is general, in a small fraction of the cells for much of the time, even during logarithmic growth. Under favourable conditions, however, most of the spores then produced re-germinate to give normal active cells and thus they do not dominate the population until a rather delicate balance of temperature, light, and nutrient depletion prevails.

The phenomenon of spore formation is well known amongst many bacteria and has been reported in some species in nearly all classes of unicellular algae; it is likely that some dormant or spore form exists for all marine species in arctic and subarctic waters.

The resting spores or *akinetes* of the Myxophyceae are very reminiscent of bacterial spores, being remarkably resistant to temperature changes and even to desiccation. The planktonic Chrysophyceae form *cysts* which may have both a reproductive and resting significance and spore formation is recorded for some unicellular Chlorophyceae, but in this class there is a great diversity of behaviour (ref. e.g. Fritsch, 1935). A senescent or spore stage in the life cycle of dinoflagellates is found in the cysts produced by many species, immobile forms with thick walls and large food reserves, capable of prolonged survival under adverse conditions. Comparatively little is known concerning the dormant forms of the Xanthophyceae (e.g. Halosphaera) but they appear to behave in some ways like the Bacillariophyceae (below).

The continuous size reduction found in succeeding generations of diatoms may lead to the eventual death of Bacillariophyceae cells. However, within a narrow size range, each species seems able to rejuvenate itself by *auxospore* formation. If this opportunity is lost, however, and the cells become too small, their progeny then have no power of ultimate survival. Auxospore formation may be comparatively rare in nature and most diatoms probably die for want of light and other reasons (Fritsch, 1935).

At all stages of growth prior to auxospore formation resting cells can be formed, with a contraction of the protoplast and a thickening of the cell wall. These spores survive adverse conditions for months or even years (ref. e.g. Allen and Nelson, 1910).

Riley (1943) found that a senile or senescent form of a marine *Nitzschia* (which suffered little or no size reduction) was produced at all stages of culturing but mainly after the exponential phase of growth. Formation of this type of cell, which could be considered to be a resting stage, was hastened in the dark. The cells were much more buoyant than normal cells and better adapted for winter survival.

When suitable conditions of light, temperature and nutrition are again encountered in the sea dormant cell forms recommence active growth, after the variable lag period described at the start of this Section.

2. INFLUENCE OF LIGHT

The rate of photosynthesis by algal cells is a function of light intensity, increasing with intensity until a "light saturation" value is reached. Above the saturation value the rate stays constant and then, if the illumination is too great, it will eventually decrease due to light inhibition of photosynthetic processes. This inhibition is brought about by photoxidation of critical enzyme systems and possibly by chlorophyll inactivation (Nielsen, 1952c). At high enough intensities there is a bleaching of the chlorophyll in algal cells and the phenomenon of *systrophism* may occur in the Bacillariophyceae, the chromatophores contracting and knotting into groups. Pigment bleaching starts with carotene, then chlorophyll and finally the xanthophylls. The reaction requires oxygen and probably occurs because of destruction of a stabilizing protein bond (Sironval and Kandler, 1958).

The absolute value of the maximum rate of photosynthesis at light saturation depends on many factors and will differ according to the way in which the crop of photosynthetic cells is measured (dry weight, cell number, amount of chlorophyll, etc.; ref. e.g. Myers, 1946a, b). It is, therefore, more useful for many purposes to express the photosynthetic rate P at light intensity I in a *relative* unit f, where f is the fraction that P is of the maximum rate of photosynthesis P_{max}. "P_{max}" occurs at the saturation light intensity I_{sat} or I_{max}:

$$f = \frac{P}{P_{max}}$$

It can be shown (ref. e.g. Rabinowitch, 1951) that all theoretical models of the role of light in photosynthesis, i.e. a rate control by enzyme reactions and diffusion processes that cannot eventually keep pace with primary photolysis, lead to the formulation of hyperbolic curves for the relation of photosynthesis to light intensity. In some circumstances these hyperbolae may be of a simple rectangular form:

$$\frac{f}{1-f} = K.I \qquad \ldots\ldots\ 17a$$

where K is a constant, but experimental points rarely fit such curves with much precision.

The empirical relation used by Smith (1937) which is of the form:

$$\frac{f}{(1 - f^2)^{\frac{1}{2}}} = K.I \qquad \ldots\ldots 18$$

has been found to hold quite well by other workers. Winokur (1949) used the equation for *Chlorella vulgaris* cultures and Talling (1957a) claimed great success with both cultured and natural populations of a freshwater *Asterionella*. K^{-1}, which has the units of light intensity, is the value of I when $f = \sqrt{\frac{1}{2}}$ and acts as a useful parameter with which to express the light response of a cell.

For these equations to be meaningful the algae must be present in an adequate medium, in a condition of "logarithmic" growth. k or k' (equation 9) are then functions of I in much the same way as is f in such equations as 17a or 18.

The success or otherwise of the Smith equation and other expressions (ref. e.g. Baly, 1935) scarcely proves or disproves the basic hyperbolic nature of light response curves (P vs. I curves), as so many other experimental factors may mask the true relationship. Ryther (1956c) gives curves for marine phytoplankters that are practically linear until light saturation.

In any case, no proposed formulae account for the subsequent decrease of f found at supra-optimal light intensities, a decrease of the greatest importance in marine productivity studies, as will be described later. The present author is of the opinion that there is no substitute for the use of experimental curves, determined over a wide range of intensities, with the particular population of phytoplankters concerned. The use of a simple linear relation as employed by Riley (1946) is to be discouraged as it misrepresents behaviour at high light intensities and can only be considered as successful, in practice, when the broad limits of precision used by ecologists are assumed to be adequate.

The units in which I has been expressed in the literature are so varied that they are best reduced to a common system. Strickland (1958), amongst others, has suggested the use of langlies per minute (ly/min) in the wavelength range 3800-7200A and has given conversion factors. It is often difficult to evaluate these conversion factors with much accuracy but errors thus introduced in the values for radiation are of little importance; the illumination conditions used by the original authors can rarely be reproducible with any better precision by another worker.

The existence of an optimum light intensity for planktonic algae had been realized for several decades. The much-quoted values given by Schreiber (1927) for a marine diatom (0.01 ly/min for *Bidulphia mobilienses*) and a marine chlorophyte (0.02 ly/min for *Carteria* sp.) now seem rather low. Jenkins (1937) found linearity between P and I with illuminations of up to about 0.06 ly/min when using *Coscinodiscus excentricus* and *Bidulphia regia*. Inhibition and systrophism set in at near to 0.16 ly/min. Wassink and Kersten (1945) showed

0.08 ly/min to be the saturation level for *Nitzschia dissipata*. Talling (1957a), working with *Asterionella* at 16°C, recorded a plateau between about 0.09 and 0.2 ly/min. Recently Kain and Fogg (1958) gave the optimum light for *Asterionella japonica* as about 0.03 ly/min but no inhibition was found with an illumination of up to 0.06 ly/min.

Turning now to natural marine populations, Nielsen (1937b), using a natural population taken from northern waters in October, reported a linear relation until about 0.03 ly/min. The curve flattened until 0.1 ly/min, after which inhibition occurred. Near Bergen in May, the rate of photosynthesis by the endemic population increased with light intensity until a maximum at about 0.06 ly/min (Berge, 1957). Nielsen and Al Kholy (1956) recorded 0.14 ly/min as the I_{sat} value for a mixed phytoplankton population in the west Pacific (see also other data by Nielsen and Jensen, 1957; Nielsen, 1957a, and Doty, 1957; where the I_{sat} value is around 0.1-0.14 ly/min.)

Ryther in an interesting paper (1956c) has divided the marine phytoplankton into three groups; Chlorophyceae, Bacillariophyceae, and Dinophyceae. At about 20°C, the growth rate versus light intensity curve showed a linear increase up to 0.03 ly/min in the first group, 0.07 ly/min for diatoms and 0.16 ly/min for the dinoflagellates. A subsequent increase of illumination then caused a continuous and almost linear decrease in photosynthesis rate with the Chlorophyta. Photosynthesis was only a few per cent of the maximum at 0.5 ly/min. The Bacillariophyceae had a fairly contant production rate between about 0.07 and 0.15 ly/min, but the rate then decreased rapidly and was again only a few per cent of the maximum at 0.5 ly/min. The Dinophyceae, on the other hand, were more resistant and still photosynthesized at some 40 to 50% of their maximum rate when the radiant energy was 0.5 ly/min. Ryther suggested a suitably weighted curve to represent the behaviour of temperate-sea phytoplankton but clearly the exact shape of such a curve must vary according to the predominant species present in a mixed population and will be characteristic of the sea area and time of year (Berge, 1957). For example, the curve for a mixed surface phytoplankton in the tropics (Nielsen and Jensen, 1957) was well to the right of even Ryther's P vs. I curve for dinoflagellates. (Samples taken from lower in the euphotic zone had a lower I_{max}).

It is perhaps worthy of mention that a paper by Sorokin and Kozlyaninov (1957) appears to imply that the *in-situ* P vs. I curves for marine phytoplankters in the Japanese sea are linear in character from the surface down. This suggests a considerable adaptation by the organisms to the changes of light intensity with depth and has not been reported elsewhere (see, however, Doty (1957) where an even more startling adaptation is implied). In a later paper Sorokin *et al.* (1959) report data for Atlantic waters where there is, in effect, a maximum in the P vs I curve. It is not clear why there is this difference in behaviour between the Atlantic Ocean and the Japanese Sea.

It is to be regreted that no response curves have been reported for the Chrysophyceae or Xanthophyceae in cultures, as these classes of algae may often constitute a significant fraction of the marine population. By contrast there are numerous papers on the Chlorophyceae, notably *Chlorella*, but the significance of work with *Chlorella* to marine studies is not very clear.

The optimum light level for this genus is low (around 0.04 ly/min at 10–15°C, ref. e.g. Phillips and Myers, quoted by Kraus, 1956; Noddack and Kopp, 1940; Myers, 1946b) and puts *Chlorella* in conformity with the Chlorophyta studied by Ryther (1956c). However, there are many complexities in the behaviour of *Chlorella*. Some of these may be worth a brief mention as similar complexities could conceivably be found also with marine phytoplankters were these organisms ever to be studied in such detail.

For example, the P vs. I curves for *Chlorella* depend greatly on the previous illumination history of the cells. Winokur (1948b) found that the P vs. I curves had a greater slope and the P_{max} was greater when the cells had been reared at high illumination rather than at low illumination. Winokur (1949) also found that the Smith equation applied to his culture of *Chlorella vulgaris* (Columbia strain) only if the cultures were under 2 weeks old. The slope increased with the age of culture and supra-optimal light inhibition became more marked. A similar behaviour is reported by Myers (1946b), although the P_{max} *per unit dry weight of cell* was stated by him to be greatest for cultures preconditioned at low illuminations. These findings of Winokur and Myers are to be compared with those of Tamiya *et al.* (1953, 1955) working with *Chlorella ellipsoidea*. This alga, when reared at high illumination, changed mainly to a "light" form (cf. Noddack and Kopp, 1940), the cells of which were much less light sensitive in their photosynthesis than were the "dark" cells which prevailed in cultures grown at a low illumination.

Myers and Burr (1940), in a study of the effect of intense illumination on *Chlorella pyrenoidosa* and *Chlorella vulgaris*, found, in effect, that P vs. I curves were strictly reproducible only with illuminations of up to about 0.03 ly/min. At greater intensities P increased (up to the I_{max} of around 0.06 ly/min) but the rate decreased with the time of exposure to light and by 0.1 ly/min or more it was constant for only a few minutes. At intensities of severe photo-inhibition (several tenths of a langley per minute) the rate was not only low initially, but rapidly decreased with further exposure and the cells became bleached. Photautoxidation was superimposed upon normal photosynthesis until it eventually exceeded the rate of respiratory oxidation. Under these conditions there was severe chlorophyll bleaching and irreversible damage could be done to the cells.

Clearly, behaviour such as this must be borne in mind whenever P vs. I curves are determined for natural populations. Ryther (1956c), who has made the most complete study to date with marine phytoplankters of known genera, used cultures pregrown at relatively low light intensities (near to 0.05 ly/min). He then preconditioned them for about 2 hours at a given I value before this point was determined on the P vs. I curve. Such a procedure appears to be about as satisfactory as can be hoped for when constructing a response curve to be broadly applicable under natural marine conditions although the exact shape of any curve will undoutedly depend upon the depth from which the population is drawn (a function of its pre-history of illumination, ref. e.g. Nielsen and Hansen, 1959; Ryther and Menzel, 1959).

The effect of temperature on the rate of photosynthesis and on the influence of light is complex. The subject is reviewed briefly by Strickland (1958). Under favourable growth conditions P_{max} often increases logarithmically with temperature over a restricted temperature range (generally between about 5° and 20°C), the quotient of a 10-degree temperature rise, Q_{10}, being 2 or a little greater. The shape of a P vs. I curve changes with temperature, becoming less linear and having progressively less slope as the temperature increases. This generally results in a marked increase of I_{max} with temperature. A logarithmic increase of (K^{-1}) with temperature in equations such as 17a or 18 fits some data very well (ref. e.g. Noddack and Kopp, 1940; Talling, 1957a; Wright, 1959).

Nothing has been said about the effect on photosynthesis of the spectral quality of the light used. There will undoubtedly be some loss of efficiency in green light as compared with other wavelengths, especially with the Chlorophyceae. Spectral effects in a natural population are complex but probably relatively small and it is customary to assume that, on balance, all light between about 3800A and 7200A is equally effective (ref. e.g. the discussion by Strickland, 1958).

3. Periodicity Phenomena in Phytoplankton Photosynthesis

A diurnal fluctuation in the rate of photosynthesis by a plant in its natural surroundings is to be expected, owing to fluctuations in the light intensity to which it is exposed. However, there may also be diurnal or similar variations in the intrinsic photosynthetic activity or "vitality" of the plant itself, i.e. its productivity index (see page 101) may change throughout a relatively short period. These changes become a matter of concern in primary productivity measurement as we may need to measure the photosynthetic rate of an algal population at constant light intensity in some form of a light "incubator". It is important to know whether or not the rate thus recorded depends upon the time of day of the experiment.

A well marked pattern of changing photosynthetic ability has been discovered in many land and higher aquatic plants, which show the phenomena of a "midday rest" or "afternoon nap", etc. (ref. e.g. Rabinowitch, 1951). This behaviour can be attributed to several factors, such as closure of the stomata, choking of the photosynthetic apparatus by carbohydrates or narcosis by the accumulation of half-oxidized products. By contrast, the simple unicellular algae, such as we find in the marine phytoplankton, can be grown in culture for many days or weeks with no notable daily periodicity in growth rate after an initial adjustment period. It may not be possible to remove all fluctuations (ref. Goryunova and Nasonova, 1955) but they can be greatly reduced by using suitable techniques, especially when working at low illuminations.

In nature, however, a pronounced diurnal fluctuation of photosynthetic efficiency has been found for marine phytoplankters. Doty and Oguri (1957) reported that the $^{14}CO_2$ uptake rate of a natural population taken from tropical surface waters varied when the bottles containing the phytoplankters were illuminated for short periods under artificial light of constant intensity. Plants taken from the sea at 0800 hours local time and placed in an incubator under an illumination intensity of 1500 ft-candles (say around 0.09 ly/min) took up $^{14}CO_2$ nearly 6 times as fast as did the same population when removed from the sea at 1900 hours local time. A similar sort of behaviour was recorded with samples taken from the sea at 20 m depth and with samples stored on the deck in carboys. Doty (1957, 1958) reported that the magnitude of these fluctuations decreased the further north one went from the equatorial region and diurnal variation ceased altogether at about 60° - 70° north. It can be predicted in any location by a graphical method based on the mean daily behaviour of a mixed population (Doty, 1958).

In accordance with these observations Shimada (1958) found a 3- to 4-fold diurnal fluctuation (maximum at 0600 to 0800 hours, minimum at 1600 hours) in the waters one mile from the shores of a Pacific island at 18° N (see also Holmes with Haxo, 1958), whereas at Woods Hole (42° N) the variation was only 2-fold (Yentsch and Ryther, 1957), although at approximately the same times (0800 hours and 2400 hours local time).

If the carbon-14 method is measuring gross photosynthesis less some substantial fraction of respiration (ref. Section IV.A) then these diurnal variations could be attributed, in part, to changes in respiration rate. However, the magnitudes are such as to make it almost certain that we are witnessing a true photosynthetic rate change. This conclusion was also reached by Verduin (1957) who has produced evidence for photosynthetic periodicity occurring in a freshwater lake. There appears to be no periodicity in the carbon-14 uptake of darkened bottles (Doty, 1958).

A rythmic pattern in the metabolic processes of many organisms is becoming increasingly apparent (see e.g. Bunning, 1956; Brown *et al.*, 1957; Harker, 1958; and references they cite) but we need not necessarily assume that the comparatively simple behaviour of the algae is always related to the same elusive factors as are operative with terrestrial plants and animals. Some narcosis or "sugar glut" is not impossible in growing phytoplankton but a more simple explanation lies in the action of light itself, probably via pigment activation in the algal cells.

Thus both Yentsch and Ryther (1957) and Shimada (1958) report a diurnal periodicity in the amount of chlorophyll in a plankton population which parallels, almost exactly, its photosynthetic activity and which can be attributed only in part to a population increase. The surface phytoplankton in summer is subjected to supra-optimal illumination and, as mentioned earlier in this section, such excess illumination can result in decreasing photosynthetic activity and loss of chlorophyll. Yentsch and Ryther (1957) found that the chlorophyll in the water of Woods Hole harbour commenced to increase at night, after several hours of darkness, and then climbed rapidly to its maximum under the photosynthetic action of the first few hours of morning light, before bleaching by full sunlight set in during the rest of the day.

This explanation has been criticized (Verduin, 1957) on the grounds that Doty found the same diurnal fluctuations to occur with 20-m samples as with those collected at the surface. However, the fluctuations at 20 m appear to be rather less marked than at the surface and in tropical seas the light at 20 m may be at least 25% of the surface illumination and could still be appreciably supra-optimal. Nevertheless, the point is well taken and further experimentation is required to settle matters . Probably several factors are operative. For example, Collier *et al.* (1953) have produced strong evidence for a diurnal fluctuation of soluble carbohydrate-like matter in sea water. The daily cycle of this material is not unlike the cycle of photosynthetic activity and points to the possibility of there being metabolic regulation by promoters and inhibitors excreted into the water by the phytoplankters themselves (ref. e.g. the review by Saunders, 1957 and recent experiments on the action of algal hormones on marine phytoplankters described by Bentley, 1958).

Hastings and Sweeney (1958) report a persistent diurnal rhythm in the bioluminescence of the marine dinophyceae *Gonyaulax polyedra*, which is independent of illumination provided that this is not too great. A similar rhythm in the cell division of this organism is found (Sweeney and Hastings, 1958) and most recently (Hastings and Sweeney, 1959) its photosynthetic potential has been shown to follow a like pattern. The behaviour is unrelated to chlorophyll content and is independent of (low) light intensity. Truly endogenous fluctuations of biochemical origin are thus possible in at least some marine phytoplankters. Doty (1957) found the periodicity effect in a natural population of tropical origin continued for several days when the population was removed from the sea, although exposure to natural daylight was necessary to maintain the effect beyond one or two days.

C. PRODUCTIVITY MEASUREMENT IN NATURE

1. Further Observations on Population Changes in Nature; Initiation of Phytoplankton Blooms, etc.

a. The initiation of spring phytoplankton blooms

Apart from considerations of a true growth lag (see earlier) the phytoplankton crop in arctic and sub-arctic waters will be prevented from "blooming" in winter by purely physical causes. The absence of sufficient radiation in mid-winter was long realized to be an inhibitory factor (cf. Atkins, 1928) but there is sufficient light on bright December days at mid latitudes to promote vigorous growth in the top meter or so of water. The reason that blooms are not then initiated, except in most exceptional circumstances, must be attributed to lack of stability in the water. Phytoplankton cells are removed from the shallow zone of adequate illumination by hydrodynamic forces before cell division can take place.

Instability in the surface water must not exceed a critical depth if the gross photosynthesis is to exceed the respiration. Gran and Braarud (1935) suggested that this critical depth should be less than about 5 times the "compensation depth" (depth of the euphotic zone).

The subject of the initiation of spring diatom flowering was treated in a semi-quantitative manner by Riley (1942). By considering the plant pigment content of the surface water and the stability of the water column over Georges Bank in March, April and May he found no correlation until the stability was positive. The rate of phytoplankton increase was very approximately:

$$\text{rate of increase} = k.\ \frac{D_E}{D_M} - \text{respiration} \qquad \ldots\ldots\ 19$$

where D_E is the depth of the euphotic zone and D_M is the depth of the mixed layer of turbulent water where the stability is zero or negative.

As we are generally concerned only with the upper 100 m of the ocean the "stability" can be expressed in the form $9.5 \times 10^{-3} \frac{\delta\sigma_t}{\delta z}$ (sec^{-2}); where z is in meters; but, bearing in mind the precision of temperature and salinity measurements, the stability should exceed about $\frac{10^{-4}}{\delta z}$ sec^{-2} before it can be considered to be positive with any certainty. It does not follow that wind-mixing will disturb all water in this layer of zero stability, but as the average Ekman depth of frictional resistance will exceed 50 m for much of the winter in most temperate latitudes, the mixing of phytoplankton throughout the unstable zone is not too unreasonable an assumption.

Sverdrup (1953) extended the above concepts in a classical paper on the vernal blooming of phytoplankton. The phytoplankters (as in Riley's paper) were assumed to be distributed evenly in a mixed zone of zero or low stability, the depth of which, D_M, had not to exceed a certain critical depth, D_c. This depth, D_c, was taken as the depth where the integral photosynthesis in a column D_c was calculated making the assumption that the phytoplankton respired at a uniform rate throughout the water column, a rate which (by definition) was equal to the rate of photosynthesis at the compensation light intensity, I_{comp}. A further basic assumption (not strictly correct) was made, namely that photosynthesis at depth z was everywhere proportional to the light intensity, I_z at that depth. This reasoning led to an expression for the critical depth:

$$D_c = (1 - 10^{-\kappa D_c}) \times \frac{1}{2.3} \times \frac{I_{surface}}{I_{comp}} \qquad \ldots\ldots\ 20$$

In equation 20, κ is the vertical extinction coefficient (see Strickland, 1958) for light of wave lengths between 4200A and 5600A and is assumed to be constant with depth. $I_{surface}$ and I_{comp} apply also to this spectral range and $I_{surface}$ may be approximated to 20% of the total surface radiation in ly/min, after correcting for reflection losses etc. I_{comp} was given a value of 0.15 ly/hour.

D_M and D_c were measured at Weathership "M" in 1949, and the phytoplankton and zooplankton were recorded in the water that spring. The time of the onset of the vernal blooming agreed quite well with prediction. D_M was shown to be about 5 times D_E (the euphotic zone depth) when D_c exceeded D_M (c.f. the suggestion by Gran and Braarud, 1935).

It will be shown later that, if the light attenuation properties of sea water are characterized by the vertical extinction coefficient at 4300A, then integration factors, R, can be calculated which enable the gross primary production *below unit area* of sea surface to be derived from a knowledge of the production *per unit volume* at the optimum light intensity, I_{max}. R values vary with $I_{surface}$ and can themselves be integrated throughout the hours of daylight to give a factor, R_{day}, which converts the production per unit volume per hour, at intensity I_{max}, to the gross daily production below unit area in the euphotic zone.

It can be shown that for production to exceed respiration in a water column of depth D_M, then D_M must not exceed D_c which is given by:

$$D_c = R_{day} \times F \qquad \ldots\ldots\ 21$$

where

$$F = \frac{I_{max}}{I_{comp} \times 24}$$

I_{max} should be taken at the top of the linear portion of P vs. I curves (see earlier in this section) and will have value between about 0.07 and 0.12 ly/min. I_{comp} in this treatment and Sverdrup's treatment should be the hourly not the mean daily value (ref. Strickland, 1958) and is unfortunately not known with any precision. It probably lies in the range 0.003 to 0.005 ly/min (Strickland, 1958) and F can thus have a value anywhere between about 0.6 and 1.6 with about 0.8 as the most likely figure. The uncertainties in equation 21 are no greater than those implicit in equation 20 and in all treatments the main errors arise from the lack of any precise values for respiration, either directly or via the term $I_{comp.}$

It is clear that in order to offset any losses by grazing and to get logarithmic growth progressing rapidly, D_c should exceed D_M by a significant amount (say 10% or more of the latter). As early spring blooms in temperate seas are nearly always comprised of diatoms with *k* values of around 0.05 hour^{-1}, favourable conditions must prevail for at least 2 or 3 days if the occurrence of a vernal outburst is to become obvious.

The above treatments may appear to be so speculative as to have little practical importance. As yet the writer has seen very little work designed to test the exactness of their predictions (e.g., Marshall, 1958), but the Sverdrup or similar calculations may well be of practical advantage in alerting biologists to the onset of primary food production and may help in assessing the duration of any true growth lag period that occurs in a natural marine environment.

No quantitative treatment is yet possible when considering the blooms that occur after the first major spring outburst. Processes of nutrient mixing followed by bright still weather are the obvious causes in many instances. However, physical conditions may be favourable and yet the rapid flagellate blooms of late summer will not occur until after what appears to be a true growth lag, during which the sea water adjusts to the exacting nutrient requirements of each species. Various coloured "tides" of flagellates have been the most studied and the reader is referred to Conover (1954), Margalef (1956) and Bainbridge (1957) amongst many others for quantitative and semi-quantitative accounts. Discrete and nutrient rich water masses are normally required which (as will be mentioned later) must exceed a certain critical size. The blooming of one species paves the way for the blooming of another by changing the level of inorganic nutrients and providing, via excretion or decay, the essential metabolites needed by succeeding organism (ref. e.g. McVeigh and Brown, 1954; Ryther, 1954b; Lucas, 1956; Margalef, 1956; Saunders, 1957; Bentley, 1958; but note also Talling, 1957c).

b. DISSIPATION OF PHYTOPLANKTON BLOOMS

Spring blooms are initiated when factors tending to remove the phytoplankton from a suitable growth environment (the euphotic zone) are offset by the reproductive processes of the plants. This is a special and simplified case of a general condition which can be expressed:

Net production rate (see Section I) = Net photosynthesis − (losses by grazing + losses by sinking + losses by eddy diffusion out of a suitable environment) 22

(Changes brought about by lateral *advection* of water masses in and out of an area under study are so complex that no generalized considerations are of much value.)

As mentioned in the introduction, we are not concerned in this review with the details of grazing and other losses but it is useful to outline the mechanism of these losses before considering the measurement of primary productivity in nature.

Grazing is generally supposed, for simplicity, to result from indiscriminate filter feeding and hence the rate of grazing at any instant is proportional to the standing crop of both phytoplankton and zooplankton. If the zooplankton concentration Z at any instant is found and the grazing coefficient G is known (the volume of water filtered per unit concentration of zooplankton in unit time) then the grazing per unit time (hour) is given by (Z.G.p). If the concentration of zooplankton is expressed in grams wet weight (or cc wet volume) per 1000 m^3, G is of the order of 10^{-5} m^3/hour but this estimation is *extremely* crude and the exact value will depend greatly on variables such as temperature and the nature of the zooplankters (ref. Fleming, 1939a; Riley, 1946; Riley *et al.*, 1949; Gauld, 1951; Marshall and Orr, 1955, 1956; Riley *et al.*, 1956; Steel, 1956, 1957a; Lasker quoted by Holmes, 1957; Cushing, 1955, 1957c; and data quoted by Cushing, 1957d, 1959).

Fleming (1939a) expressed the loss by grazing in terms of the initial loss at time zero, which can be written (Z.G.*p*) and an additional time dependent term, (Z′.G.*p*.*t*) where Z′.*t* expresses the increase of a predatory zooplankton population with time. If the mean division rate of the plant population is known then Z.G and Z′.G, can be evaluated from the population profile. The Fleming equation gives a useful model of the effect of grazing on population dynamics and predictions made using this equation were in broad agreement with data taken from Harvey *et al.* (1935). However, changes in phytoplankton population found in nature can rarely be attributed solely to the effect of grazing superimposed on logarithmic growth, even although grazing may at times be the dominant factor.

Losses will also occur at any point in the euphotic zone by plant cells falling down through the water under gravity, a process which takes place with all cells except, perhaps, for some species of flagellates and Myxophyceae when these are at the peak of their metabolic efficiency. Losses due to sinking can be expressed:

$$\frac{-\partial p}{\partial t} = 10^{-2}.V_{T}.\frac{\partial p}{\partial z} \qquad \ldots\ldots\ 23$$

where V_T is a sinking velocity in cm/hour, t is in hours and z is measured in meters (positive from the surface downwards). To allow for the effect of temperature a Stoke's law correction can be made:

$$V_T = V_{0}.\frac{\mu_0}{\mu_T} \qquad \ldots\ldots\ 24$$

where V_0 is the sinking velocity at 0°C, and μ_0 and μ_T the viscosity coefficients of sea water at 0°C and T°C respectively (ref. Riley *et al.*, 1949).

V_0 will depend on the shape and size of the individual phytoplankters and their density. The density of Bacillariophyceae may sometimes be as much as one or two units in sigma-*t* less than the surrounding water but once past the peak of metabolic efficiency cells become appreciably more dense than the surrounding water and can sink at the rate of many metres a day. A range for V_0 of about 5 to 25 cm/hour with the most likely value near to 12 cm/hour was computed for mixed populations by Riley *et al.* (1949) (see also Steel, 1956) but there is no reason to suppose that even a mixed population will have a V_0 value as constant as this range suggests. There is evidence that individual populations may sink up to 5 or 10 times more slowly or quickly (ref. e.g. Allen, 1932; Riley, 1943; Gross and Zeuthens, 1948; Steel, 1957a). V_0, like the grazing coefficient G, has so uncertain a value that generalized computations are almost meaningless and *in situ* data must be obtained if any quantitative predictions are to be attempted.

Superimposed upon grazing and sinking losses a natural phytoplankton population can also suffer dissipation (or augmentation) by turbulence carrying plant cells into or out of a favourable growth environment.

The quantitative treatment of turbulence in productivity studies has not yet advanced beyond the expedient classical approximations using "Austausch" coefficients. As there is confusion in some texts as to the definition and interrelation of such coefficients (which we will here call "virtual coefficients") they will be defined for references:

i. Virtual diffusion coefficient A_d

$$\text{Flux of substance} = A_d \times \text{concentration gradient}$$

In terms of a Fick-type constant, D_d of dimensions $L^2.T^{-1}$;

$A_d = D_d$; *dimensions*: $L^2.T^{-1}$; *units*: cm^{2}sec^{-1}

ii. Virtual heat conduction coefficient A_h

$$\text{Flux of heat} = A_h \times \text{temperature gradient}$$

In terms of a Fick-type constant, D_h:

$A_h = \rho.C_p.D_h$; *dimensions*: cals.M.L^{-1}.T^{-1}; *units*: cal.g.cm^{-1}.sec^{-1}.

(where ρ is the density of sea water in g./cc and C_p is its specific heat).

iii. Virtual viscosity coefficient A_μ

$$\text{Flux of momentum} = A_\mu \times \text{velocity gradient.}$$

In terms of a Fick-type constant, D_μ;

$A_\mu = \rho.D_\mu$; *dimensions*: M.L.$^{-1}$.T^{-1}; *units*: g.cm^{-1}. sec.$^{-1}$.

These three different D values (cm^{-2}sec^{-1}) are not necessarily the same numerically, as the processes governing the transfer of dispersed substances, heat or momentum differ from each other in the sea. However, D_d and D_h can perhaps be equated without serious error, as C_p and ρ for sea water approximate to unity in the c.g.s. system. This equality is important in work, described in the last part of this Section, concerned with the transport of nutrients. The values for D_d and D_μ, however can vary widely (by an order of magnitude or greater) and the indiscriminate use of a symbol A (no subscript) and the implied assumption that $\frac{A}{\rho}$ (probably $\frac{A_\mu}{\rho}$), governs material transport is to be discouraged.

If we consider the dispersal of a phytoplankton crop by turbulence we may write:

$$\frac{\partial p}{\partial t} = 0.36\ A_d . \frac{\partial^2 p}{\partial z^2} \qquad \ldots\ldots\ 25$$

for a vertical plane and similar expressions, with the corresponding A_d values, for dispersal in other directions. Again z is in meters and t in hours for phytoplankton work whereas A_d is kept in the normal units of $cm^2 sec^{-1}$.

The variability of A_d in both time and space makes the application of equation 25 very difficult, even in the open ocean, and the treatment is, for all practical purposes, meaningless in coastal waters. A_d may have a value ranging from less than unity to many hundred $cm^2 sec^{-1}$, according to the water stability and the tidal or wind driven currents, and it will vary markedly with depth. The virtual coefficient is not independent of time, except for periods of a few hours or, at most, a few days. In the Sargasso Sea, where the population was assumed to be approximately constant over a period of several days A_d values were estimated by Riley *et al.* (1949) but the results depended on a simultaneous knowledge of V_0 and, *at best,* indicated an order of magnitude.

If we assume that the Fleming type of grazing equation and equations 23, 24 and 25 apply simultaneously and if we use the growth constant *k'*, defined by equation 8, to govern ***net*** photosynthesis, then, when ***biological transport*** can be neglected, the ***net production rate*** (equation 22) is given by the following expression:

$$\frac{\partial p}{\partial t} = p\left[k' - G(Z + Z'.t)\right] - V_o \frac{\mu_e}{\mu_T} . \frac{\partial p 10^{-2}}{\partial z} + 0.36 . A_d . \frac{\partial^2 p}{\partial z^2} \qquad \ldots\ldots\ 26$$

If the biotic factors (the first term on the right hand side of equation 26) are simplified to a time-independent term C.*p*, this equation is of the form:

$$A\left(\frac{d^2 p}{dz^2}\right) + B\left(\frac{dp}{dz}\right) + C(p) = \frac{dp}{dt}$$

where A, B and C are constants. For the simple case where the population is stationary or is changing at a constant rate the treatment is standard for a second order differential equation. Riley *et al.* (1949) have presented a formal treatment of this problem when $\frac{dp}{dt} \rightarrow 0$ but the approximations necessary to establish boundary conditions are so extreme as to render any application of the solution of doubtful value, except perhaps in tropical waters of high stability, and even then agreement with observation may be fortuitous. Steel (1956) has used a similar form to equation 26 to predict g and V_0 from known values of $\frac{dp}{dt}$. Recently Cushing (1959) has presented a very readable treatment of plankton population dynamics with heavy stress on grazing aspects, which he considers probably dominate the picture for much of the year.

A semi-quantitative treatment of the dissipation of plant cells by turbulence has some value when considering the horizontal dissipation of blooms, in particular the flagellate blooms that appear in northern waters towards the end of summer.

These organisms have exacting nutrient requirements and will not produce their characteristically intense blooms or "tides" unless the dissipation rate from a biotically suitable body of water into an unsuitable environment is less than the reproductive rate of the phytoplankton cells. As many Dinophyceae are capable of phototactic movement and can be concentrated by week convergences we have what is, essentially, a surface problem with fairly uniform light conditions and hence a constant mean value for k'. Losses are significant from the horisontal plane only, often in only one or two main directions.

The problem was treated by Kierstead and Slobodkin (1953, see also Slobodkin in the paper which follows) by solving the equation:

$$\frac{\partial p}{\partial t} = 0.36A. \frac{\partial^2 p}{\partial x^2} + k'.p \qquad \ldots\ldots 27$$

with the assumption that only one main direction of loss was involved. Boundary conditions are now relatively simple and precise as $p \rightarrow 0$ when x is either zero or some critical length, L metres. The solution is then of the form:

$$L = B\left[\frac{A_d}{k'}\right]^{\frac{1}{2}} \qquad \ldots\ldots 28$$

where B is a constant, depending on the shape of the water mass, and is in the range of about 2 to 4 for most systems. L is the length across the bloom in the direction of the most rapid dissipation (ideally the only direction of dissipation). For long thin blooms, such as are often observed stretching for many hundreds of yards in slicks of weak convergence, B is equal to 2. Assuming $k' = 0.1$ hour^{-1} a suitable environment would have to exceed a length of 100 m were A_d equal to $2.5 \times 10^2 cm^2 sec^{-1}$. The size corresponding to an A_d of $2.5 \times 10^4 cm^2 sec^{-1}$ would be 1000 m. Similarly for a roughly circular patch, where B is about 3, suitable water masses must exceed 150 or 1500 m in diameter when A_d is 2.5×10^2 or $2.5 \times 10^4 cm^2 sec^{-1}$, respectively. These predictions are not unreasonable, bearing in mind what is commonly observed in calm summer days and in strongly stabilized water (ref. also Ragotzkie and Pomeroy, 1957), but we cannot ignore considerations of the velocity, length and time scales of turbulent disturbances (ref. e.g. Stommel, 1949). A direct test of the application of these equations in confined areas might perhaps be attempted by making a simultaneous determination of the horizontal A_4, using a dye dispersion technique (e.g. Moon *et al.*, 1957).

Further considerations of the complex relationship between the biotic levels in the ocean is outside the scope of this review and the reader is referred to the numerous reviews and texts on this subject (ref. e.g. Volterra, 1928, 1931; Kostitzin, 1939; Alee *et al.*, 1949; Riley, 1953a, etc.) for details and a consideration of the various types of approach.

It will be noted that in equations such as 26 and 27 the growth constant is designated by k' (equation 8) rather than by k (equation 4). The latter, for a given temperature and illumination, implies an environment that imposes no growth limitations due to inadequate nutrients or the presence of toxins. In nature this ideal state will rarely be encountered and a parameter k' is more appropriate. The relationship between k' and k assumes considerable importance when attempting quantitative ecological predictions after the manner of Riley (1946, 1947) and Riley *et al.* (1949) but we have at present no precise knowledge of the relationship even under the simplest conditions of nutrient deficiency.

It seems pointless, at present, to attempt the evaluation of k' from the integrated form of any of the differential equations suggested above, the parameters and boundary states are too variable and too difficult to establish. The interrelation of k' and k, however, is of great interest, especially when considered in conjunction with a full chemical and physical analysis of the environment, and should be measured if we are ever to obtain a realistic assessment of the effect of nutrients on *in situ* phytoplankton growth. There seems to be no reason why this cannot be attempted by a direct determination of the differential, $\frac{\delta p}{\delta t}$, especially with the relatively rapid $^{14}CO_2$ technique now available. If measurements are made in a light incubator with water that has been filtered to remove the bulk of grazers then equations such as 9 and 13 can be studied directly by sampling daily throughout the growth and decline of (preferably) a monomictic bloom. If sufficient direct rate determinations are possible then the difference between the observed population and the population predicted by graphical integration of the observed rate data would give a relatively precise estimate of the total losses by grazing and other factors.

C. THE "LAND-MASS" EFFECT

Before leaving a discussion of phytoplankton growth patterns in nature, brief mention should be made of the effect of the proximity of land on marine phytoplankton growth, the "land-mass" effect. It is generally recognized that inshore waters, especially over the continental shelf or near oceanic islands, support a greater crop of marine plants and animal life than is to be expected in the open ocean, even when the nutrient content appears to be similar, (e.g. Gran, 1932; Sverdrup *et al.*, 1942; Hart, 1942; Brodsky and Vinogradov, 1957;

and many others) but quantitative comparisons are rare and it is not clear whether or not the net primary productivity as well as the primary product is greater at inshore locations.

As the bottom topography of most coastal waters favours upwelling and tidal mixing a more sustained crop yield might be expected at the coast than in the less turbulent regions of the open ocean but the main question is whether or not the photosynthetic rate at any given time is greater near land than further out to sea. There is recent evidence that this may be so (Doty, 1955; Doty and Oguri, 1956) as the net primary productivity (as measured by $^{14}CO_2$ uptake) was found to be one or two orders of magnitude greater a mile or less from shore than at stations 15 miles or more from the coast.

The most obvious explanation of this is that the proximity of land introduces favourable organic or inorganic growth substances into the water, either via land drainage water or because of the nearness of the sea bed with its benthic population and reserves of particulate inorganic material.

However, only the PI (productivity index) will indicate whether or not one body of water is inherently more or less productive than another. In this regard the observations by Currie (1957) are of the greatest interest. The present author has seen no other published PI values which are so directly related to this problem, although the general crop and productivity picture in some other sea areas is in general agreement with Currie's results (e.g. Holmes, 1957).

Currie, using total pigment as a crop measure, found that the PI in the northeast Atlantic was almost the same close to the Iberian coast as at stations 100-200 miles out to sea and yet in the latter locations the standing crop was an order of magnitude less. This state of affairs persisted over a period of several weeks. The pronounced difference in the standing crop density in these two areas was not, therefore, attributable to any marked difference in mean k' values. Such a conclusion, if substantiated for other parts of the world, is of the greatest significance for comparative marine productivity research and further experimentation is urgently needed. The only likely explanation involves differences in either grazing, sinking and turbulent dispersion, or, as Currie suggested, perhaps the excretory losses of combined carbon in the open ocean may greatly exceed similar losses by a neritic crop. The respiration rates of the two crops could also differ and this might have a noticeable effect as the $^{14}CO_2$ method is probably measuring a rate somewhat greater than the net rate of photosynthesis. The mean temperature at the oceanic stations was several degrees higher than at the coast.

Finally, it may be worthwhile to repeat the warning made on page 95. In shallow areas (less than 25-75 m, depending on the water transparency) the primary productivity of the water proper should not be judged solely by changes in its dissolved oxygen or carbon dioxide contents or by the amount of benthic fauna that appears to be sustained beneath its surface. There may well be an intensely active but closed ecological system on the bottom sands and muds with a high photosynthetic potential, quite independent of the over-riding sea water (ref. e.g. Kohn and Helfrich, 1957; Oliver, 1958; Odum and Hoskins, 1959; Pomeroy, 1959).

2. *In situ* PRODUCTION AND ITS PREDICTION FROM EXPERIMENTS IN LIGHT INCUBATORS

a. DIRECT MEASUREMENT OF *in situ* PRODUCTION

The most obvious method for determining the *in situ* primary productivity in a column of water is to enclose suitable volumes of water in clear glass or plastic containers and suspend these containers at the depths from which the samples originated. The photosynthesis is then measured by oxygen evolution or carbon dioxide assimilation over a suitable period of time (ref. Section IV).

This technique (using only surface water) was tried out by Gaarder and Gran (1927) and thereafter by numerous workers who generally employed the L and DB method on water returned to the depth of sampling (ref. e.g. Marshall and Orr, 1928; Gran and Thompson, 1930; Neilsen, 1932; Clarke and Ostler, 1934; Neilsen, 1937b; Jenkins, 1937; Riley, 1938a; Neilsen, 1951a; Talling, 1957a). More recently radiocarbon uptake experiments have been used to measure *in situ* photosynthesis (ref. e.g. Neilsen, 1952a; Miyake *et al.*, 1954; Berge, 1957; Wimpenny, 1957; Currie, 1957; Steel, 1957a; Jitts, 1957; Holmes, *et al.*, 1957; Ryther and Yentsch, 1958).

In some instances the suspension of these bottles in the euphotic zone has been made directly from the side of a research vessel or from the end of a pier but it is more normal to use a buoy of metal or glass with the string of bottles, or certainly the bottles in the first few meters, held away from the buoy by some form of boom. Details of the types of arrangement used may be found in many papers (e.g. Gaarder and Gran, 1927; Marshall and Orr, 1928, 1930; Gran and Thompson, 1930; Neilsen, 1937b; Jenkins, 1937; Levring, 1947; Wimpenny, 1957; Currie, 1957; Holmes, 1957; Talling, 1957a). Free floating buoys may be recognized by marking with a flag (Holmes *et al.*, 1957) or, more ingeniously, by erecting a radar reflector (Currie, 1957). In the latter case the ship is released for a day or half a day to work in other locations. To enable surface samples to be measured whilst the ship is in motion these may be trailed astern, just beneath the surface (Holmes *et al.*, 1957).

It is general to enclose samples in 100- to 300-ml capacity glass bottles clipped directly to a weighted wire or rope or, if many samples are used at each depth, held in some form of tray (Jenkins, 1937). Jitts (1957), to minimize any disturbance to the phytoplankton, sampled the water in adjacent light and dark lucite sampling bottles and returned them to the euphotic zone without any transfers. To ensure adequate mixing in the bottles a small piece of glass rod or a few glass beads may be inserted (Currie, 1957) but this seems scarcely necessary under most natural marine conditions and there may be little need for mixing during exposure of a few hours (Talling, 1957a).

The exposure times used with the *in situ* technique vary in the literature from periods of many days using the L and DB technique (Riley, 1938a) down to a few hours when working with radiocarbon. The most usual period is a full 12 or 24 hours for L and DB oxygen measurements and a half day for $^{14}CO_2$ uptake experiments. In the latter case a period from either sunrise to mid-day or mid-day to sunset is favoured. With short exposure times it is important that bottles be filled and inocculated with radioactive carbonate, etc., as rapidly as possible in very subdued light. For measurements made at the bottom of the euphotic zone on a bright day the rate of raising and lowering the samples and the time of overside handling can assume considerable practical importance.

The most serious problem in translating results obtained from a string of bottles to the true natural production concerns the duration of the experiment. In bright summer weather the supra-optimal light at the top of the euphotic zone has the effect of slowly reducing the productivity index (see earlier in this Section) and as a result the sum of say six 2-hour periods of exposure may predict several hundred percent more phytosynthesis than is found in one continuous 12-hour experiment. Rohde (1956, 1957) and Verduin (1957) have stressed the importance of this effect which can, however, vary greatly, being much less on dull days and with plants having a high I_{max}. It is not clear what the true *in situ* photosynthesis will be near the surface in bright sunlight. Phytoplankton cells are not without vertical movement during a period of several hours and a given cell may not be retained for the whole time at one depth, as it is in a glass bottle. The true productivity is likely to be greater in nature than would be inferred from a bottle experiment lasting all day but probably not as great as the sum of several exposures of a few hours each.

Short exposures have the further disadvantage that extrapolation to the full 24 hours production becomes progressively less precise the briefer the experimental period (see Doty, 1958). Periodicity effects are also more serious and a daily production based on the results of a few hours exposure around 0800 hours may well exceed the prediction based on a similar period in the late afternoon, even if the light intensities throughout the day are relatively constant.

b. RELATION OF *in situ* PRODUCTION TO PRODUCTION MEASURED IN A LIGHT INCUBATOR

Despite the attractiveness of *in situ* productivity measurement, which is probably the most reliable method yet devised for estimating the total rate of photosynthesis in the euphotic zone, the method is very limited in its application.

Except at inshore stations, situated conveniently near a marine laboratory, or at a weathership or lightship the technique is costly and inconvenient and is useless when the productivity of a wide sea area is to be measured on cruises where little ship delay is permissible. It is therefore necessary to devise some form of shipboard technique which can be applied to samples withdrawn from the euphotic zone when the ship is on station for hydrographic or similar measurements. To this end various methods of incubating samples in tanks under artificial or natural illumination have been devised.

These methods can be divided into two main categories. In the first the samples are exposed to daylight but are covered with various filters, neutral grey or coloured, so as to reduce the light intensity to the value expected at a known depth in the sea. A series of samples from the euphotic zone can then be incubated under conditions of illumination that approximate to their natural environment and hence a productivity profile is obtained. This is probably the most satisfactory method of using a light incubator and the shortcomings are mainly associated with difficulties of construction which will be considered later. The agreement between results using this method and direct measurements in the euphotic zone appears to be quite good (Berge, 1957; Cushing, 1957b, c).

In the second category, samples from all depths (or a composite sample from the euphotic zone) are incubated for a known period at one light intensity. Such a method, using either sunlight or artificial daylight lamps, has been very popular in assessing the *relative* productivity of surface waters during expeditions (e.g. Riley, 1939b, 1941b; Riley and Gorgy, 1948; Neilsen, 1952a; King *et al.*, 1957; Holmes *et al.*, 1957; Neilsen and Jensen, 1957; and others). However, the relative data so obtained are limited in value and it would be much better to use a constant light intensity incubator to predict production in the whole euphotic zone. Such a prediction appears to be feasible provided that certain experimental relationships are first determined.

If sea water samples containing phytoplankton are exposed for a known time to the optimal light intensity (I_{max}) of the population concerned, then the maximum possible primary productivity (P_{max}) may be obtained in terms of mg C/m^3/hour. Suppose that the *relative* P vs. I curve (the f vs. I curve, ref. page 115) is known for this population over a wide range of photosynthetically active light intensities and that the amount of this photosynthetically effective radiation (about half the total radiant energy recorded by a pyrheliometer) can be measured at the sea surface throughout the day. Also, let us assume that the light attenuation properties of the sea in the area under study are known sufficiently well for one to estimate the fraction of the surface radiant energy present at any given depth. With this information we may calculate the absolute value of I at any depth (I_z) and from the f vs. I curve we may thus find the fraction of P_{max} (viz. f) that applies to this depth. Now f will be unity at a depth where I_z happens to equal I_{max} and will be less than unity at greater or lesser distances from the surface. Thus in sufficiently bright daylight the maximum photosynthesis will occur some distance beneath the sea surface and the point of maximum photosynthesis will move upwards as the light decreases, reaching the surface only when the illumination is quite poor (0.1–0.3 ly/min of *total* radiant energy as compared with a mid-day summer value of 1.4 ly/min or more).

Thus, if we know the f vs. I curve of our phytoplankton population and the light attenuation properties of the water, we can construct a series of profiles of f vs. the depth (z metres) for each assumed value of the surface radiation. These curves are taken to a depth where I_z reaches I_{comp} and the integral of each curve gives a factor R which relates the incubator production P_{max} (at I_{max}) to the total production in the euphotic zone thus:

$$\text{Production beneath unit area per hour} = R \times \text{Production per unit volume per hour at } I_{max} \quad \ldots\ldots 29$$

or

$$P_{E.hour} \text{ (in mg C/m}^2\text{/hour)} = R \times P_{max} \text{ (in mg C/m}^3\text{/hour)}$$

"R" varies with the surface radiation and the light attenuation properties of the water but for each type of water (see later) it can be graphed against the ly/min of photosynthetically active surface daylight. Because of the depressing effect of photosynthesis in the surface waters by supra-optimal light R increases relatively slowly with increasing $I_{surface}$ values when these are greater than about 0.4–0.5 ly/min (0.8–1.0 ly/min total energy) and may even pass through a flat maximum. If values of R are read from a graph, using the *mean* surface radiation level for each hour throughout a day, then the integral of this plot of R against hours of daylight gives a new factor, R_{day}, which now converts the production per hour at maximum light intensity (P_{max}) to the *total daily production in the euphotic zone* thus:

$$\text{Production beneath unit area per day} = R_{day} \times \text{production per unit volume per hour at } I_{max} \quad \ldots\ldots 30$$

or

$$P_{E.day} \text{ (in mg C/m}^2\text{/day)} = R_{day} \times P_{max} \text{ (in mg C/m}^3\text{/hour)}$$

The primary productivity predicted by these formulae will be either gross or net according to whether or not the R factors are calculated from f vs. I curves showing f in terms of gross or net photosynthesis. In the latter case f, and hence R, values will be negative beneath an I value of I_{comp}, but we are not computing for depths exceeding the compensation depth.

The above treatment is theoretically sound and the general shape of the production profile with depth which it predicts has been confirmed experimentally by a number of workers (e.g. Marshall and Orr, 1928; Jenkins, 1937; Neilsen, 1955a, 1957a, b; Ryther, 1956c; Rodhe, 1957; Currie, 1957). The main source of error is probably associated with variations in the exact shape of f vs. I curves. Ideally, the curves should be determined for every phytoplankton population being studied at every depth but such a procedure is clearly impractical. When working in sub-arctic waters we can calculate R from some form of averaged temperate zone curve such as that given by Ryther (1956c). However, trial calculations show that in the extreme cases of either pure Bacillariophyceae or pure Dinophyceae population, R is appreciably different, especially at low light levels. As *f* vs. I curves are to some extent affected by the exact design of light incubator it may be best to determine these curves directly for each incubator used and this determination should be repeated several times a year. If such a program is impractical then the published P vs. I curves for a comparable latitude and time of year can be used, but there will be a loss of accuracy. The "*in situ*" determination of *f* vs. I described later is probably the best compromise.

When the euphotic zone coincides with a layer of near-isothermal well mixed water a single P vs I curve is probably applicable. However, evidence is accumulating (Nielsen and Hansen, 1959; Ryther and Menzel, 1959) that thermally stratified waters may contain several populations with markedly different P vs I relationships due to an acclimatization to different light or temperature conditions or to the presence of different species at different depths. Algae at the base of the euphotic zone have much lower I_{max} values than those near the top. Any attempt to predict productivity profiles by the use of several *f* vs I curves becomes so complicated that it would be better abandoned in favour of the direct incubation of samples at various light intensities mentioned earlier.

The other main problem concerns the measurement of the light attenuation properties of sea water, which is best carried out by some simple measurement made at the time of taking productivity samples for incubation.

A complete profile of photosynthetically active light can be made by finding the vertical extinction coefficients of several wave bands of light (Jenkins, 1937) but this is tedious and time consuming. At the other extreme workers have taken a simple κ value (ref. Strickland, 1958) to apply for the attenuation of all photosynthetically active radiation, but such a procedure is so crude that some compromise appears necessary. It is suggested (present author) that use could be made of the Jerlov water types (Jerlov, 1951). A complete radiation attenuation pattern has been reported by Jerlov for the water in 12 different coastal and oceanic sea areas, the water masses being characterized by a κ value obtained at any one wave length, wavelengths between about 4300A and 4800A giving the most sensitive differentiation. It appears likely that, for the precision required in productivity studies, any sea area outside the influence of quite abnormal land drainage can be "typed" by measuring its κ (4300A) value. R vs. $I_{surface}$ curves are then constructed using the Jerlov data for the attenuation of submarine light in the type of water concerned.

Ryther (1956c) has treated the problem somewhat differently by assuming a single coefficient κ to apply to the photosynthetically active light in a water column. R may then be obtained in terms of a "relative light depth" which is measured in units of $\kappa.z$ (as $I_z = I_{surface}\ \exp.(-\kappa.z)$) so that for any location:

$$P_{R.day} = \frac{R'_{day}}{\varepsilon} \times P_{max} \qquad \ldots\ldots 31$$

"R'_{day}" was calculated from weekly averages of the daily radiation throughout the year. (The R'_{day} values given by Ryther are based on an absorption coefficient, naperian logarithms, and should be multiplied by 0.434 if the more convenient extinction coefficient (κ), from base-10 logarithms, is used). Equation 31 was used by Ryther and Yentsch (1957) to calculate the gross primary productivity, over a wide sea area from chlorophyll data, assuming the relation:

$$P_{max} = 3.7 \times (\text{mg chlorophyll/m}^3)$$

This subject has already been discussed in Section IV.G.

In the above discussion it has been assumed that the phytoplankton population is distributed uniformly in the euphotic zone. This is often not the case, even in tropical waters, as suggested by the profiles given by Holmes *et al.* (1957), Neilsen (1952a) and Neilsen and Hansen (1959). Neglecting to allow for such variations may introduce considerable errors. R or R_{day} factors should therefore be evaluated for depth increments of 5 or 10 m down to the bottom of the euphotic zone and used for samples withdrawn at the mid points of these depth increments.

A method advocated by Sorokin (1956, 1959) may be adapted to give what is probably the most satisfactory "incubator" technique. The Sorokin procedure takes into account the uneven distribution of phytoplankters with depth by finding the *relative* productivity of samples taken at intervals throughout the euphotic zone. These samples, including a surface sample, are exposed for a suitable interval in a light incubator at a light intensity that may have any value so long as it is in the semi-proportional region below I_{max}. At the same time a duplicate surface sample is exposed at the sea surface, either from a buoy or trailed from the ship. This sample serves to fix the absolute rate of productivity as affected by the incoming solar radiation. A third sample, which may also be from the surface, is used to find what is, in effect, the f vs. I response for the population under study. This latter determination is made by exposing aliquots at various depths where the light intensity has known values and is repeated each time the population or light attenuation characteristics of the water undergo a marked change.

The method is well suited to open ocean work on expeditions where lengthy stations can be taken (Coblenz-Mishke, 1958) and is essentially similar to the integration factor approach described above, using multi-depth R values, except that R values are not actually calculated and f vs. I curves are determined using *in situ* I values.

In the opinion of the present writer it is this latter aspect of the procedure which is most important, as such a method is probably the most practical and satisfactory way to obtain a f vs. I relationship. The experiment should be carried out around mid-day in fine clear weather and the light intensity at each depth *must* be calculated using the same method as that later employed when determining I_e during the calculation of R (e.g. the κ (4300A) measurement suggested above). In this way many errors and uncertainties in the evaluation of R or R_{day} will cancel each other. As a final refinement the P_{max} value determined by the light incubator can be normalized by a factor to make it equal the P_{max} value found *in situ*.

There are as yet, unfortunately, very few precise comparisons published of *in situ* productivity data and the results of calculations made from constant light incubator experiments and integration factors.

Nielsen (1952a, 1957a) has presented an empirical formula which gives quite a satisfactory prediction of natural productivity if constants are obtained for each location and each incubator used. The equation is of the form:

$$P_{K.day} = \tfrac{1}{5}\,(2.P_{100} + 2.P_{10} + P_1) \times \frac{D}{2} \times N \times K \;\ldots\ldots\; 32$$

where the P values are the incubator productivity values measured with a sample taken from the surface (P_{100}) from a depth where the photosynthetically active light intensity is 10% (P_{10}) and where the light is 1% of the surface value (P_1). D is the depth in metres where the light is 1% of the surface value and N is the day length in hours. K is a constant which depends on the incubator, sea location and the precise incubation light intensity (which need not be quite as great as I_{max}). In the tropics Nielsen (1952a) found a K of about 1.0 when samples were illuminated at 18,000 lux. Another treatment used by Rohde (1956) for lakes leads to similar results. Results obtained with this formula and the Ryther formula for temperate waters (equation 31) are in fair agreement in the tropics but part of this agreement must be fortuitous as the phytoplankters almost certainly have different f vs. I curves in the two locations. Results quoted by Corlett (1957), using an appropriate Nielsen equation for sub-arctic regions, agreed very well with *in situ* measurements but predictions by the Ryther equation were low. The Nielsen equation has much to recommend it on the grounds of simplicity and the use of a simple empirical approach may be all that is worthwhile in the light of present experimental techniques (cf. Verduin, 1957).

At the other extreme Talling (1957b) has presented a purely mathematical treatment of the photosynthesis-depth integral, assuming the Smith equation (equation 18) to apply. The f vs. I curves were not taken to supra-optimal light intensities, which invalidates the use of his equations in marine work, except for rather dull weather or turbid waters. The assumptions made in the Talling treatment are rather extreme but the agreement between experiment and theory turned out to be quite reasonable for the particular case of an isothermal lake in spring. However, attempts to generalize such methods are of doubtful practical value in view of the mathematical complexity of any final expression which attempts to include all natural variables.

c. THE DESIGN OF INCUBATORS AND THEIR USE

Certain aspects of the design and use of light incubators merit brief discussion. In all cases samples must be cooled by running water or the addition of ice so that the temperature approximates to the temperature of the euphotic zone, or at least to its surface. This aspect of design is relatively simple and requires no further mention. The main problems arise from the provision of suitable illumination and the correct use of the incubation technique.

Let us consider first the first type of incubator, where we require the illumination of the sample in the incubator to be equal to the illumination found at the depth from which the sample was drawn. This is most simply achieved by using an open-topped tank, receiving full daylight, and covering samples with neutral grey filters to reduce the light to certain known fractions of the surface illumination (ref. e.g. Berge 1957). However, under these conditions the spectral characteristics of the light are quite unrepresentative and it is much better to attempt to augment neutral filters wih coloured glass plates that allow only the correct wavelengths of light to pass. The exact filter combination will, of course, depend on the depth under consideration, but some approximation should be attempted (Cushing, 1957b, c; Nielsen, 1957a). Jerlov (1954) has suggested filter combinations which give approximately the same transmission as one meter of the various water types found in coastal and oceanic regions. These filters could be used in conjunction with neutral grey filters or even directly, although in the latter case the cost would be considerable. Finally, rather than rely on natural illumination a combination of neutral and coloured filters can be used in conjunction with an artificial light source (ref. e.g. Cushing, 1957c). Jerlov (1954) has recommended filters for changing the light from an incandescent tungsten source to give the spectral distribution of the light from the sun in a blue sky.

There have been several designs used for incubators intended to illuminate samples at a constant light intensity. Considering the present state of the art, there is little cause for assuming one type of design radically better than another. In general, samples are enclosed in a glass or clear plastic container through which water is circulated and which is illuminated by strip fluorescent lamps (Doty, 1955, 1957; King *et al.*, 1957; Jitts, 1957). To raise the level of illumination the lamps may be backed by reflecting surfaces and the tank material may be contructed of polished metal or painted white. By this means the amount of light received by a bottle can be increased by up to 50%. To ensure even illumination the carriage holding the sample bottles may be rotated (Nielsen, 1952a; Corlett, 1957) or may be surrounded by circular fluorescent lighting (Doty, 1955).

Perhaps the best design of incubator is one in which an attempt is made to imitate natural conditions, i.e. lights are fitted onto a lid and shine down uniformly (through some form of diffusing screen) onto bottles which rest on a dark background. Jenkins (1937) and Cushing (1957c) report experiments where samples are incubated both in clear bottles and in flat sided bottles which have only the tcp surface clear, the others being blackened. Both authors agree that the production for a given light source is *greater* in bottles lighted solely from the top. However, Jenkins reported that this behaviour occurred only at *supra-optimal light intensities*, where it could be explained, in part, by the fact that algal cells in the clear bottles received more light, and hence inhibition, than those in the partly darkened bottles. Cushing, on the other hand, claims that the effect is found at all levels of illumination. The matter merits further study.

The vertical illuminating of bottles in dark or dull surroundings has the advantage that the total illumination received by the phytoplankters can be measured more precisely, in fact a pyrheliometer can be used in the tank to standardize the equipment directly in ly/min. If a judicious mixture of blue and green fluorescent lighting is used (rather than daylight lamps) much long wavelength radiation is avoided. The light energy is practically all in the photosynthetic range, with a spectral distribution not unlike that of light a few meters below the sea surface. The output of fluorescent tube lighting slowly decreases in efficiency and lamps should be replaced after a few hundred hours burning time.

It is not clear to what extent agitation is necessary when samples are being incubated (Talling, 1957a). For periods of only a few hours, such as are used for radiocarbon work, there would seem to be little need for stirring or shaking (Doty, 1957, 1958).

Many of the precautions that must be observed with *in situ* productivity experiments apply equally well to work in incubators. The period of illumination should be kept as long as possible and samples taken from beneath the surface should be shielded from direct daylight whilst they are transferred to and from the incubator. The periodicity effect must be borne in mind and, in this regard, it is best not to sample either early in the morning or late in the afternoon or else samples should be kept, exposed to light, until some suitable standard time (ref. Doty, 1958).

One problem that arises with constant light incubator work that is not encountered when working with samples *in situ* is the need for preconditioning the algae. Plant cells taken from deep in the euphotic zone and placed immediately into an illumination approaching I_{max} may well have a different photosynthetic efficiency and P vs I relationship than they would were they back in the sea (ref. Nielsen and Hansen, 1959). There is no simple way in which this difference can be overcome or allowed for but fortunately the contribution of such cells to the total productivity is not large. Changes of pressure have been shown to have little effect (Doty, 1958) but there is still a need for further investigation (ref. e.g. Doty, 1957).

Finally it should be noted that we have no knowledge yet of what may be the effect of the submarine polarization of light on photosynthetic productivity. McLeod (1957) has demonstrated the importance of the degree of circular polarization on the rate of photosynthesis. Both plane polarization (ref. e.g. Waterman and Westell, 1956) and elliptical polarization (Ivanoff and Waterman, 1959) is known to occur in the sea. There will be negligible polarization in an incubator lighted with fluorescent tube lamps.

All zooplankton should be removed before measuring primary productivity and this can be done adequately with a coarse net (ref. Section IV). A technique such as that used by Jitts (1957) where the bottle used for taking a sample in the euphotic zone is transferred directly to an incubator suffers from the disadvantage that the inclusion of several zooplankters could go undetected. The presence of even one or two adult copepods per 100 ml can have a marked effect on the apparent productivity (Cushing, 1957c).

3. *In situ* PRODUCTION AND ITS PREDICTION FROM CHANGES IN THE CHEMICAL COMPOSITION OF NATURAL WATERS

In theory the net productivity of a column of water may be deduced from the change, with time, of the concentration of biotically active elements, notably carbon, oxygen, phosphorus and nitrogen. Ideally such a method is perhaps the most satisfactory approach to the problem of marine productivity as it can give results integrated over comparatively long periods of time and over wide sea areas. This removes most of the uncertainties attached to "instantaneous" measurements when applied to such a heterogeneous system as marine phytoplankton.

Early estimates of the production during the spring and early summer were made by several workers based on the changes of pH, oxygen or phosphorus in a shallow column of sea water (ref. e.g. Atkins, 1922 and 1923; Buch, 1929; Kreps and Verbinskaya, 1930 and 1932; Cooper, 1938a; see also Armstrong and Harvey, 1950; Amstrong, 1954; Riley *et al.*, 1956; and references in Gaarder and Gran, 1927). The "base line" from which changes are measured is generally the mid-winter values for the water column or, in the case of oxygen, the saturation concentrations at atmospheric pressure. As a refinement Buch (1929) suggested that the "initial" oxygen could be estimated from the concentration of nitrogen gas, assuming that both elements were originally in equilibrium with the air.

When assessing productivity, oxygen changes are perhaps the least ambiguous, although total carbon dioxide data would be as satisfactory were it not for analytical difficulties. Both these determinations can be used to estimate the net productivity in terms of carbon with little error due to conversion factors, as these are comparatively precise. However, interchange of oxygen or carbon dioxide with the atmosphere can give rise to some uncertainty (ref. Steel, 1957c, and the technique used in shallow waters by Odum and Hoskins, 1958). The measurement of the concentrations of nitrogen, silicon or phosphorus avoids this last difficulty but the conversion of these elements into the equivalent of combined carbon is much less precise (ref. Section I.B).

Nitrogen analyses do not appear to have been used (see however Riley 1951a), mainly because of technical difficulties. Unfortunately silicon changes, although easy to measure, do not reflect the production of all phytoplankton, although, as Atkins (1928) has pointed out, a comparison of the variations of silicon and phosphorus concentrations in a column of water should give an indication of the relative growth of silicified and non-silicified phytoplankters (the Chrysophyta compared with the other divisions). We are left, therefore, with phosphorus as the only element, other than oxygen, to find much favour as a production indicator. All living material incorporates this element and its analysis, at least in the inorganic form, is comparatively simple.

Two major uncertainties prevent the measurement of oxygen or phosphorus profile changes from being a precise indicator of the productivity in a water column. Firstly, we have to consider regeneration problems and secondly, and more important, there are complications arising from the lateral transport of oxygen or phosphorus into or away from the area under study. When considering only the upper layers of a water column vertical transport also becomes significant.

Regeneration problems do not affect the interpretation of oxygen data if we consider changes of oxygen concentration to represent the *net* production of organic matter, gains being by photosynthesis and losses either by direct or respiratory oxidation. Any net gain, however, cannot be equated to an increase in *living* phytoplankton or even to particulate material; the gain merely represents the formation of organic matter not degraded back to carbon dioxide but, as such, may well provide a realistic estimate of much of the increased food potential in a water mass. Seiwell (1935) has equated the annual *loss* of oxygen in a water column of oceanic depth with the annual *production* of organic matter, assuming the oxygen was utilized in oxidizing all photosynthesized organic material. Photosynthetically liberated oxygen in the surface layers was assumed to be without effect. These assumptions are rather extreme (ref. Riley, 1951a).

When considering the changes in inorganic phosphorus content (or for that matter inorganic nitrogen as nitrate) regeneration presents a more serious complication. It has already been mentioned (Section IV.H) that a large fraction of the total soluble phosphorus in sea water can be organically combined and that much of this organic phosphorus may be directly available for plant synthesis. Thus changes of orthophosphate content can be very misleading, except perhaps in arctic and sub-arctic waters after the winter period of remineralization. The changes of mineralized phosphate concentration will, of course, indicate the net production of organically combined phosphorus. However, this production is not necessarily the same as the long term production of plant material, as measured by the L and DB or other techniques. It is clear that the liberation of phosphorus (all forms) from plankton can take place at quite different rates than the gross oxidation of organic matter as reflected by oxygen consumption (see for example the highly variable ratios reported by workers such as Riley, 1951a). Organic phosphorus, liberated from cells or detritus by hydrolytic or partially oxidative mechanisms, is again available for further plant synthesis. When considering phosphorus changes, therefore, we should measure the changes of *total soluble* phosphorus, which is a comparatively lengthy and tedious analysis, and even this measurement only approximates to the synthesis of living plant material in the time interval concerned.

Uncertainties arising from the vertical and lateral transport of oxygen and phosphorus can be of either a hydrodynamic or biotic nature. The only transport so far considered quantitatively in open sea productivity work is the vertical mixing of nutrients by turbulence. This is important when studying the net productivity at any one level in the sea or the changes in the upper portion of a water column of oceanic depth. (In the open ocean only changes in the upper layers are significant for direct productivity measurement. The whole water column contains so much oxygen or phosphorus in its deeper layers that short term changes of biotic origin are an insignificant fraction of the total).

Using the nomenclature and definitions given earlier in this section (page 126), we may write the following equation for the vertical conservation of an element at a depth z meters:

$$\frac{\partial c}{\partial t} = \frac{\partial}{\partial z}\left(A_d.\frac{\partial c}{\partial z}\right) - K.\frac{\partial p}{\partial t} \qquad \ldots\ldots 33$$

where c is the concentration of an element such as oxygen or phosphorus in mg/m^3 and K a constant converting the primary product (p) in mg C/m^3 to the equivalent concentration of the element represented by c. Rewriting in terms of D_d (equal to A_d):

$$\frac{\partial p}{\partial t} = \frac{D_d}{K}.\frac{\partial\left(\frac{\partial c}{\partial z}\right)}{\partial z} - \frac{1}{K}\frac{\partial c}{\partial t} \qquad \ldots\ldots 34$$

Considering now the transport of a conservative property such as heat, we may write for a temperature change ($\partial\theta$):

$$\frac{\partial\theta}{\partial t} = \frac{\partial\,(\text{heat flux})}{\partial z}$$

whence from the definitions given earlier in this section:

$$\frac{\partial\theta}{\partial t} = C_p.\rho.D_h.\frac{\partial\left(\frac{\partial\theta}{\partial z}\right)}{\partial z} \qquad \ldots\ldots 35$$

If we now assume that D_h is equal to D_d, that is the transfer of heat and dissolved substances occur by the same mechanism, and that the D coefficients are constant throughout all (small) depth intervals under study we may write:

$$\frac{\partial p}{\partial t} = \frac{1}{C_p.\rho.K} \cdot \frac{\partial \theta \, c_{(zz)}}{\partial t \, \theta_{(zz)}} - \frac{1}{K} \cdot \frac{\partial c}{\partial t} \qquad \ldots\ldots 36$$

where $C_{(zz)}$ and $\theta_{(zz)}$ are the second differentials of concentration and temperature with depth at the depth z. If the subscripts 1 and 2 apply to the values for p and c at a depth z at two different times, t_1 and t_2, the above equation in terms of *finite differences* over a short time interval $(t_2 - t_1)$ becomes:

$$p_2 - p_1 \cong \frac{(\theta_2 - \theta_1)}{K} \cdot \frac{\bar{c}_{(z+\delta z)} + \bar{c}_{(z-\delta z)} - 2\bar{c}_{(z)}}{\bar{\theta}_{(z+\delta z)} + \bar{\theta}_{(z-\delta z)} - 2\bar{\theta}_{(z)}} - \frac{c_2 - c_1}{K} \qquad \ldots\ldots 37$$

where δz is a small suitable depth interval (say 5 to 20 m) and $\bar{c}$ and $\bar{\theta}$ are the *mean* values for c and θ at depths $(z + \delta z)$, $(z - \delta z)$ and z over the small time interval concerned. C_p and ρ are assumed equal to unity. The productivity $\frac{p_2 - p_1}{t_2 - t_1}$ can therefore be evaluated at each depth and the total productivity is then obtained by graphical integration to the required depth. An analogous expression can be used in which salinity replaces temperature but the latter is probably more convenient to measure. Calculations should not be made for the first few meters at the surface where temperature or salinity is most directly affected by radiant energy or precipitation and evaporation. The precision of equation 37 will decrease the longer the time interval $t_2 - t_1$ but this is offset to some extent by taking the mean values for c and θ over this time interval which should be chosen as a compromise between theoretical dictates and practical considerations of the precision of measuring $(\theta_2 - \theta_1)$, $(c_2 - c_1)$, etc. In the extreme case where $(\theta_2 - \theta_1)$ is zero the productivity is directly equivalent to concentration changes. Where concentrations do not change over a time interval, indicating a quasi-steady state, the productivity is just balanced by the turbulent transfer of nutrients.

This method of estimating productivity by calculating the net nutrient depletion in surface waters was pioneered by Riley. The much disputed productivity of the Sargasso sea was estimated by Riley and Gorgy (1948) using oxygen data and working on the assumption that a steady-state existed in the oxygen profile $\frac{dc}{dt} \rightarrow 0$. The temperature data used in this work were so inadequate that the present writer questions whether the resulting productivity value can be accepted even as an order of magnitude. Much of this criticism was anticipated by Riley who published a more thorough treatment in 1951 (Riley, 1951a). Recently Riley (1957) has presented a detailed study of new Sargasso data. Here the transport in relatively small depth increments was assessed. Individual A_d values were calculated for each depth range assuming that the coefficient was constant below 300 m and had a value of 0.6 cm^2sec^{-1} (see Riley, 1951b).

The method has also been applied by Steel (1956, 1957b) to productivity studies in the Fladen Ground area of the North Sea. An equation similar to equation 37 was used although *the present author cannot understand* the finite difference approximation to $\frac{c_{(m)}}{\theta_{(m)}}$ which was used. Results were evaluated from temperature and inorganic phosphorus data and were compared with radiocarbon production figures (Steel, 1957a; see also his discussion in 1957b). The agreement between both methods was considered by Steel to be acceptable and he stressed the complementary nature of the two procedures, one showing long-term and the other short-term productivity. The agreement of some individual depth-productivity profiles left much to be desired.

This approach to primary productivity estimation is open to heavy criticism, although it must be said, in all fairness to its chief exponents, that most of this criticism was anticipated in the original publications. Apart from difficulties due to regeneration, already discussed, a serious problem arises from the temporal variation of temperature (or salinity) profiles. The presence of internal waves of short period may make a temperature profile, even in the open ocean, subject to hourly variations (e.g. Defant, 1950; Rudnick and Cochran, 1951). Results at Weathership "P" (50° N, 145° W) obtained by Tabata (1958) show that changes of 1.5°C or greater may occur in the temperature at a given depth over a period of a few hours. The effect is most marked near to density gradients and appears to result from the passage of internal waves of a tidal character. Although Steel and Riley realized the possibility of errors from this source they have presented no data to determine the magnitude of the effect which can clearly be very serious.

In the opinion of the present writer productivity calculations of this type should not be attempted unless at least four temperature (or salinity) profiles are taken throughout a 24-hour period at the beginning and at the end of the time interval $t_2 - t_1$ (the latter being of the order of at least a week). The four temperatures thus recorded at each depth should be averaged to give the final data used in equation 37. By the same criterion we probably ought to mean oxygen or phosphorus data over a diurnal cycle but the magnitude of such variations in oceanic waters is at present unknown. The extra work required for such refinements would not be prohibitive, especially on a cruise which had primary productivity measurements as one of its major objectives, and it is to be regretted that no attempt has yet been made to test the method with really adequate data.

Before leaving the question of the vertical transport of nutrients into the euphotic zone the possibility of a vertical transfer by biotic agencies should be metioned. The respiration and excretion of migrating zooplankters, in particular the diurnally migrating species which enter and leave the surface layers of the sea from great depths, may well produce significant changes of oxygen or phosphorus concentrations over a period of several weeks. The magnitude of such changes, when compared with the results of turbulence or photosynthesis, is not yet known but it could be appreciable. A decade ago Sewell and Fage (1948) drew attention to this possibility in connection with the oceanic oxygen minimum layer and the reader is referred to Armstrong and Harvey (1950) for remarks on phosphorus changes attributable to zooplankton migrations.

However, the most serious criticism that can be levelled at attempts to estimate productivity from *in situ* nutrient changes concerns errors introduced by the *lateral* movement of water into and out of the column or layer under study either by turbulence or advection.

Quite recently Cooper (1957) has admitted the impossibility of using nutrient data from single stations in the Channel during the last decade or so. Several water masses of different origin drift in and out of the location making vertical profile changes almost meaningless (see also Armstrong and Harvey, 1950; Armstrong, 1954; etc.). Cooper implied that conditions may have been more favourable in the 1920's when some of the pioneer work by Atkins was carried out.

The location of Fladen Ground, used by Steel for his studies, appears to be more suitable. Here there was a relatively stable pool of deep water and any surface movements were thought to transport water of very similar nutrient and heat content into the region. Even at Fladen, however, Steel (1956, and 1957b) found anomalies which appear to arise from lateral disturbances. Unpublished results of nutrient profiles at Weathership "P" (50° N, 145° W) obtained by the present author show that the method under discussion would be useless in this area of the north Pacific Ocean. Nutrient profiles changed substantially over lateral traverses of only a few miles, due to the presence of different "biotic submasses" of water. Direct measurements of productivity rates showed that such changes would have taken months to occur as a result of *in situ* photosynthesis.

Riley (1951a, b) has presented empirical and semi-empirical attempts to evaluate lateral mixing with sufficient precision for biological purposes and formally the approach is similar to that given above for vertical turbulence. The main problem, however, is one of obtaining sufficient experimental data, especially where advection processes must be taken into account. Except for a relatively few areas, where the general oceanography is known with certainty, the presence or absence of lateral movement at all relevant depths must be ascertained. This entails at least two multi-station traverses at right angles through the region under study, each traverse being extended for a distance equal to the mean water movement during the time $(t_2 - t_1)$ over which productivity is being assessed. The overside oceanographic effort, analytical program and computation is formidable and may be impracticable for one ship, even in a relatively uniform oceanic area.

In turbulent inshore waters with a complex hydrography and rapid advection the problem is probably insoluble by existing techniques and resources. Exception can be made in the case of Shallow tidal creeks (Ryther *et al.*, 1958) or bays (Odum and Hoskins, 1958; Park *et al.*, 1958) or fjord-like inlets, where the balance of conservative properties can be evaluated with some precision (ref. e.g. the review by Prichard, 1952, and Tully, 1959). Here the lateral transport is largely in one direction only and there are several ways in which it can be estimated. Certain inlets with sills near the mouth have only annual flushing by deeper waters and hence crude estimates of the possible limits of annual productivity may be attempted (Waldichuck, 1956).

Cushing (1959) has made some interesting observations on the significance of mid-winter silicon and phosphorus levels in lakes or shallow sea areas on the total production of phyto and zooplankton during the following year.

We may conclude, therefore, that for certain open ocean areas the nutrient depletion technique of productivity measurement may be feasible. The few attempts to use the method that have been reported to date are largely unsatisfactory but mainly because of lack of sufficient data for the precise correction of vertical and lateral transport and for the proper measurement of regenerative processes. However, these attempts were preliminary in nature and had to rely mainly on results obtained during other investigations. It remains to be seen how satisfactory the method may be when properly applied using adequate oceanographic resources.

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